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July 7, 2009

Moraima Ramos-Valle
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

RE: Notification of GRAS Determination for *Haematococus pluvialis* extract characterized by component astaxanthin esters (of common edible fatty acids)

Dear Ms. Ramos-Valle:

In accordance with proposed 21 CFR § 170.36 (Notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62 FR 18939-18964), I am submitting in triplicate, as the agent to the notifier, Fuji Chemical Industry Co., Ltd., a revised GRAS Notification for *Haematococus pluvialis* extract characterized by component astaxanthin esters (of common edible fatty acids) per your request in your email of July 6, 2009.

Astaxanthin esters derived from *Haematococcus pluvialis* are intended for use as a nutrient in baked goods, beverages, cereals, chewing gum, coffee and tea, dairy product analogs, frozen dairy desserts and mixes, hard candy, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and soft candy at use levels of 0.5 mg astaxanthin *per* serving (reference amounts customarily consumed, 21CFR 101.12).

Please let me know if you have any questions.

Sincerely,

(b)(6)

Edward A. Steele
President

Enclosures

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*Specializing in FDA Regulatory Matters*

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**GRAS NOTIFICATION**

## I. Claim of GRAS Status (Revised 7/7/2009)

### A. Claim of Exemption from the Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR 170.36(c)(1)

Fuji Chemical Industry Co., Ltd. (the notifier) has determined that *Haematococcus pluvialis* extract characterized by component astaxanthin esters (of common edible fatty acids) is Generally Recognized As Safe for use as a nutrient, and therefore, exempt from the requirement of premarket approval, under the conditions of its intended use as described below. The basis for this finding is described in the following sections.

Signed,

(b)(6)

Date 7/7/09

Edward A. Steele

Agent for:

Fuji Chemical Industry Co., Ltd.
55 Yokohoonji, Kamiichi,
Toyama 930-0397, Japan

## B. Name and Address of Notifier:

Mitsunori Nishida
Fuji Chemical Industry Co., Ltd.
55 Yokohoonji, Kamiichi,
Toyama 930-0397, Japan

Tel: +81-76-472-2323
Fax: +81-76-472-2330
Email: nishida@fujichemical.co.jp

## C. Common or Usual Name of the Notified Substance:

The common name of the substance of this notification is astaxanthin esters. The substance is a complex mixture, primarily of lipids, with astaxanthin esterified with common edible fatty acids as the primary carotenoid present. Astaxanthin esters provide a source of astaxanthin when ingested. For clarity in the remainder of this notice, the term astaxanthin esters is used to describe the mixture of carotenoids that is the subject of this GRAS determination and esterified astaxanthin to describe the principal component of this mixture. As described below, the composition of the commercially available astaxanthin esters may vary depending on the source and extraction process.

## D. Conditions of Use:

Astaxanthin esters derived from *Haematococcus pluvialis* are intended for use as a nutrient in Baked Goods, Beverages, Cereals, Chewing Gum, Coffee and Tea, Dairy Product Analogs, Frozen Dairy Desserts and Mixes, Hard Candy, Milk Products, Processed Fruits and Fruit Juices, Processed Vegetables and Vegetable Juices, and Soft Candy at use levels of 0.5 mg astaxanthin *per* serving (reference amounts customarily consumed, 21CFR 101.12). Estimates of possible daily intake from the intended use levels of astaxanthin-rich extract were determined using the CSFII 2003-04 (USDA, 2004) database. Based on these determinations, the intended use of the extract in the above mentioned food categories results in an estimated daily intake of astaxanthin for "users only" at mean and 90$^{th}$ percentile of 3.2 mg/person (<0.1 mg/kg body weight/day) and 5.4 mg/person (0.1 mg/kg body weight/day), respectively.

## E. Basis for GRAS Determination:

In accordance with 21 CFR 170.30, the intended use of astaxanthin esters has been determined to be generally recognized as safe (GRAS) based on scientific procedures.

There is sufficient qualitative and quantitative scientific evidence, including human and animal data, to determine safety-in-use for

astaxanthin esters. The safety determination of astaxanthin esters is based on the totality of available evidence, particularly human observations and a variety of animal, as well as *in vitro* studies, with clinical trials further corroborating its safety. In human clinical trials no adverse effects of astaxanthin were noted at doses of up to 40 mg/day. Results from a subchronic rat study show that astaxanthin esters had no adverse effects at dose levels up to 50 mg astaxanthin/kg body weight/day, the highest dose tested. In another subchronic study, no adverse effects of astaxanthin were noted at doses up to 465 and 557 mg astaxanthin/kg/day in male and female rats, respectively. The estimated daily intake of astaxanthin from the intended uses of the extract at the 90$^{th}$ percentile of 5.4 mg/person (0.1 mg/kg body weight/day) is approximately seven-fold lower than the demonstrated safe levels of intake in humans of 40 mg of astaxanthin/person *per* day (0.67 mg/kg body weight/day). Compared to the safe levels of astaxanthin determined in animal studies, the estimated daily intake in humans is over 500 to 5000-fold lower. The estimated daily intake, if ingested daily over a lifetime, is considered safe.

### F. Availability of Information:

The data and information that form the basis for this GRAS determination will be sent to U.S. Food and Drug Administration (FDA) up on request or will be available for FDA review and copying at reasonable times at the offices of:

Mitsunori Nishida
Fuji Chemical Industry Co., Ltd.
55 Yokohoonji, Kamiichi,
Toyama 930-0397, Japan
Tel: +81-76-472-2323
Fax: +81-76-472-2330
Email: nishida@fujichemical.co.jp

## II. Detailed Information About the Identity of the Notified Substance:

### A. Chemical Name

Astaxanthin. Chemically known as (3S,3'S)-3,3'-dihydroxy-beta,beta-carotene-4,4'-dione.

### B. Trade Name:

The subject of this notification will be marketed as AstaREAL®. Fuji Chemical Industry Co., Ltd. manufactures different types of products containing astaxanthin esters that are marketed under the trade name AstaREAL® as a food ingredient. Currently, these products include AstaREAL® (formerly known as AstraCarox®), AstaREAL® Oil 50F, and

AstaREAL® L10. AstaREAL® (dry biomass) which contains free and esterified astaxanthin (total of 5% in the free form as measured after hydrolysis). AstaREAL® Oil 50F, which is marketed in Japan, also contains 5% astaxanthin in its free form, and is prepared by employing an acetone extraction process. AstaREAL® L10 which contains 10% astaxanthin in its free form, is marketed in the U.S., and is prepared by employing a supercritical $CO_2$ extraction process.

## C. Chemical Abstract Registry Number:

Astaxanthin: 472-61-7. There is no CAS number for esterified astaxanthin.

## D. Chemical Formula:

The empirical formula of the astaxanthin molecule in astaxanthin esters is $C_{40}H_{52}O_4$

## E. Structure:

The structural diagram of the astaxanthin molecule is presented below. In astaxanthin esters, astaxanthin is present in its esterified form (with common edible fatty acids).

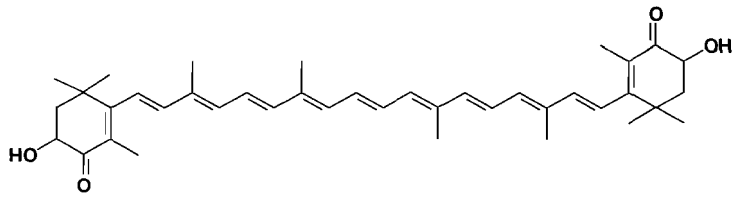



**Figure 1. Chemical structure of astaxanthin**

## F. Molecular Weight

596.8 Daltons for astaxanthin.

## G. Physical Characteristics

Astaxanthin esters are available as dark red viscous oils.

## H. Typical Composition and Specifications

Typical compositional analysis and specifications of astaxanthin esters manufactured by employing supercritical $CO_2$ extraction are presented in Tables 1 and 2. Compositional analysis of astaxanthin esters manufactured by employing an acetone extraction method is similar to that of a supercritical $CO_2$ extraction method except the acetone extracted product has higher values for tocopherols (6.1%) and phospholipids (0.2%). The slightly higher levels of tocopherol and phospholipids may be related to the polar nature of the extraction solvent acetone. The specification of

astaxanthin esters prepared by both methods is essentially the same. For the acetone extraction method an additional specification for acetone (<5 ppm) is included.

**Table 1. Compositional analysis of astaxanthin esters**

| | Typical value | Assay method |
|---|---|---|
| Acylglycerol | | |
| Triacylglycerol | 53.5 %,w/w | JFRL Method |
| Diacylglycerol | 17.7 %, w/w | |
| Monoacylglycerol | ND | |
| Subtotal | 71.5 %, w/w | |
| Free fatty acids | 0.5 %, w/w | JOCS 3.3.5; JP 2.02 |
| Carotenoids | | Reverse phase HPLC |
| Astaxanthin | 15.7 %, w/w | |
| Others* | 1.1 %, w/w | |
| Subtotal | 16.8 %, w/w | |
| Tocopherols | 2.6 %, w/w | Normal phase HPLC |
| Phospholipids | 0.042 %, w/w | JOCS 2.4.11 |
| Moisture | 0.27 | JP 2.48 |
| Total | 91.4 %**, w/w | |

JFRL = Japan Food Research Lab; JP = Japanese Pharmacopoeia; JOCS = Japan Oil Chemists' Society. *Include lutein, adonirubin, β-carotene, catnthaxanthin. **As analysis for different constituents was performed at different laboratories, the total may be less than 100%

**Table 2. Specifications for astaxanthin esters***

| | Specification | Typical value | Assay method |
|---|---|---|---|
| Description | Dark red viscous oil | Complies | Visual test |
| Astaxanthin | NLT 10% (As free form by HPLC) | 10.9% | JP 2.24 & JP 2.01 |
| Pheophorbide | Less than 20 mg/dL | Complies | Kanshoku No.99** |
| Heavy metals | Less than 10 ppm | Complies | NMKL 161 |
| Arsenic | Less than 2 ppm | Complies | NMKL 161 |
| Lead | Less than 1 ppm | Complies | NMKL 161 |
| Mercury | Less than 1 ppm | Complies | NMKL 170 |
| Cadmium | Less than 1 ppm | Complies | NMKL 161 |
| Microbiological assays | | | |
| Total plate count | Less than 1000 cfu/g | Complies | NMKL 86,4 2006 |
| Yeast and Mold | Less than 100 cfu/g | Complies | NMKL 98 |
| Coliform bacteria | Negative/g | Negative | NMKL 44, 6, 2004 |
| *Staphylococcus aureus* | Negative/g | Negative | NMKL 66 |
| *Salmonella* | Negative/g | Negative | NMKL 125,4 2005 |

*Astaxanthin esters prepared by employing acetone extraction method have an additional specification of acetone NMT 5 ppm. cfu = colony forming units; JP = Japanese Pharmacopoeia; NMKL = Nordic Committee on Food Analysis, **Notice from Environmental Health Bureau-Ministry of Health and Welfare, Japan dated May 8th, 1981

A typical fatty acid profile of astaxanthin esters is summarized in Table 3. The fatty acid profile of astaxanthin esters prepared from the supercritical CO2 extraction method or the acetone extraction method is essentially the same.

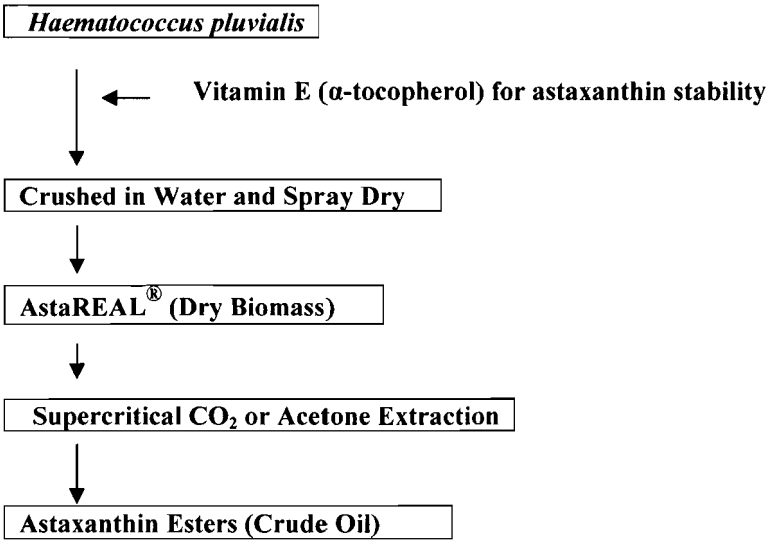
**Table 3. Fatty acid profile of astaxanthin esters**

| Fatty acids | Percentage* |
|---|---|
| C12:0 | 0.4 |
| C14:0 | 0.4 |
| C16:0 (palmitic acid) | 15.7 |
| C17:1 | 3.8 |
| C18:0 | 4.0 |
| C18:1 (oleic acid) | 30.7 |
| C18:2 (n-6) (linoleic acid) | 27.7 |
| C18:3 (n-6) | 1.2 |
| C18:3 (n-3) (linolenic acid) | 11.5 |
| C18:4 (n-3) | 1.2 |
| C20:0 | 0.7 |
| C20:1 | 0.1 |
| C20:2 (n-6) | 1.1 |
| C20:4 (n-6) | 1.0 |
| C20:5 (n-3) | 0.3 |
| C22:0 | 0.1 |
| Others | 0.1 |
| Total | 100 |

*Percent of total fatty acids

## I. Manufacturing Process

*H. pluvialis* is used as a starting material for the production of astaxanthin esters. The algae are grown in stainless steel photobioreactors under carefully controlled conditions and harvested and dried under vigorous standards of hygiene. The details of the production process are presented in the process diagram below. Food grade antioxidant (Vitamin E) is added to the product in accordance with good manufacturing practices to stabilize astaxanthin from oxidation. The algae cell walls are crushed in water and dried following addition of vitamin E. The resulting dry biomass is called biomass AstaREAL® (formerly known as AstaCarox®). The astaxanthin esters are extracted with supercritical $CO_2$ or acetone from biomass AstaREAL®. This process concentrates the lipid fraction, including the carotenoids and is essentially similar to one that is used commonly in the food industry to defat a variety of products. Proprietary information on the cultivation medium shows that it is composed of mineral salts commonly used in growth media, necessary trace nutrients, and a GRAS, food grade acid for pH control. The extracted crude oil contains esterified astaxanthin and other lipids. All processing aids used are food grade as specified in the Food Chemicals Codex. Astaxanthin esters are manufactured in accordance with current Good Manufacturing Practices (cGMP).




**Manufacturing process for astaxanthin esters**

## J. Intended Technical Effects

The intended uses of astaxanthin esters are for addition to foods as a nutrient. Astaxanthin has been shown to protect the cellular and mitochondrial membranes and ocular tissues against oxidative damage. Astaxanthin is naturally present in certain marine foods such as shellfish, crustaceans, fish, and krill. Addition of astaxanthin to certain specified foods is intended for populations that do not consume these types of food (i.e., vegetarians, vegans and certain others) and who may wish to supplement their dietary intake with astaxanthin extracted from alternative sources such as algae. The level and purported beneficial effects of astaxanthin to achieve certain health benefits has been noted in intervention trials where individuals consumed astaxanthin as a supplement to their diet.

While the use of astaxanthin esters in foods may also impart a color to food products, the intended use of astaxanthin esters would fall outside the definition of "color additive" for the following reasons: 1. The intended use levels are low enough that it dose not impart a significant color to the food products [21 CFR 70.3(f)]. 2. Astaxanthin esters are solely added for their nutritive value and thus may constitute an "unimportant color" [21 CFR 70.3(g)]. 3. The intended use of astaxanthin esters in certain specified foods is to provide consumers with an additional source of astaxanthin in their diet and does not relate to any use of the ingredient as a color

additive [21 CFR 70.3(f)]. These points are further discussed in the Expert Panel statement.

## III. Summary of the Basis for the Notifier's Determination that Astaxanthin Esters is GRAS

An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Fuji Chemical Industry to determine the Generally Recognized As Safe (GRAS) status of astaxanthin esters intended for use as a dietary nutrient. A comprehensive search of the scientific literature was also utilized for this review.

Based on a critical evaluation of the pertinent data and information summarized here, the Expert Panel members have individually and collectively determined by scientific procedures that addition of astaxanthin esters at the levels specified herein to Baked Goods, Beverages, Cereals, Chewing Gum, Coffee and Tea, Dairy Product Analogs, Frozen Dairy Desserts and Mixes, Hard Candy, Milk Products, Processed Fruits and Fruit Juices, Processed Vegetables and Vegetable Juices, and Soft Candy, and meeting the specification cited above and manufactured according to current Good Manufacturing Practice, is Generally Recognized As Safe (GRAS) under the conditions of intended use.

In coming to its decision that astaxanthin esters are GRAS, the Expert Panel relied upon supportive data that neither astaxanthin nor any of its degradation products pose any toxicological hazards or safety concerns at the intended use levels, as well as on published toxicological and clinical studies and other articles relating to the safety of the product and astaxanthin. It is also their opinion that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

IV. Basis for a Conclusion that Astaxanthin Esters is GRAS for its Intended Use.

## EXPERT PANEL STATEMENT

## DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ASTAXANTHIN ESTERS AS A NUTRIENT

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# DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ASTAXANTHIN ESTERS AS A NUTRIENT

## 1. INTRODUCTION

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel)[1], qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened by EAS Consulting Group, LLC, at the request of Fuji Chemical Industry Co, Ltd., Japan, to determine the Generally Recognized As Safe (GRAS) status of the use of astaxanthin esters as a food ingredient in Baked Goods, Beverages, Cereals, Chewing Gum, Coffee and Tea, Dairy Product Analogs, Frozen Dairy Desserts and Mixes, Hard Candy, Milk Products, Processed Fruits and Fruit Juices, Processed Vegetables and Vegetable Juices, and Soft Candy at use levels providing 0.5 mg astaxanthin *per* serving (reference amounts customarily consumed, 21CFR 101.12). A comprehensive search of the scientific literature for safety and toxicity information on astaxanthin was conducted through May 2009 and made available to the Expert Panel. The Expert Panel independently and critically evaluated materials submitted by Fuji Chemical Industry and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

### 1.1. Background

Astaxanthin [(3S,3′S)-3,3′-dihydroxy-β,β-carotene-4,4′-dione] (Figure 1) is a member of a select group of carotenoids known as xanthophylls, or oxygenated carotenoids. In contrast to β-carotene, astaxanthin possesses no provitamin A activity and, unlike some carotenoids, astaxanthin is not converted to Vitamin A in humans (Jyonouchi et al., 1995). Astaxanthin is a dietary carotenoid found in the red pigment of crustacean shells (i.e. crabs, shrimp), salmon, and the asteroidean (Miki et al., 1982). Because astaxanthin is naturally present in seafood such as salmon, red fish, shrimp, krill and lobster, it is commonly consumed by humans (Torrissen et al., 1989; Turujman et al., 1997). The pigment was first isolated in 1938 from lobster (Kuhn and Soerensen, 1938). One of the richest natural sources of astaxanthin is the green microalga *Haematococcus pluvialis*. Astaxanthin and *Haematococcus* algae meal are commonly used as color additives in the feed of salmonid fish to enhance the color of the flesh. In addition to its use as a color additive in feeds, astaxanthin has also received considerable attention for its antioxidant properties as demonstrated in both experimental and clinical studies (Hussein et al., 2006; Pashkow et al., 2008).

[1]Modeled after that described in section 201(s) of the Federal Food, Drug, and Cosmetic Act, As Amended. See also attachments (curriculum vitae) documenting the expertise of the Panel members.

## 1.2. Chemistry and Biological Activity

Chemically, astaxanthin has two chiral atoms which are at the 3 and 3′ positions of the rings (Figure 1). The two chiral centers can exist either in the R or S form, and thus there are a total of three isomers; (3S,3′S), (3R,3′S) or (3R,3′R). The (3S,3′S) and (3R,3′R) are enantiomers of opposite optical activity. The (3R,3′′S) form is an optically inactive *meso* form. Astaxanthin has several double bonds in the polyenic part of the molecule, each of which can potentially exist in the Z or E form. In nature, *Z* isomers have been observed at positions 9, 13, and 15. Thus several geometric isomers are possible: all-E, (9Z), (13Z), (15Z), (9Z,13Z), (9Z,15Z), (13Z,15Z), and (9Z,13Z,15Z). In wild Atlantic salmon, the predominant stereoisomer is (3S,3′S) which occurs as the free form (Bernhard, 1990).

While structurally similar to the familiar carotenoid β-carotene, astaxanthin has thirteen conjugated double bonds, whereas β-carotene has eleven. On the cyclohexene structure, it has oxo groups in the 4 and 4 prime positions that increase its antioxidant potential. Additionally, astaxanthin has hydroxyl groups at the 3 and 3 prime position, making the molecule somewhat polar. These structural attributes of astaxanthin have been purported to enhance its membrane function activity and thus provide a protective effect against degenerative conditions. Astaxanthin is more polar than other carotenoids but the esters are relatively non-polar. Compared to β-carotene, astaxanthin has been reported to exhibit superior antioxidant properties and is without any pro-oxidative properties (Martin et al., 1999). Available scientific literature on astaxanthin, including the results of preclinical studies and human clinical trials, suggest that astaxanthin, with its unique molecular structure and potent antioxidant activity may be effective in preventing cellular oxidative damage.

## 1.3. Description, Manufacturing Process and Specifications

General descriptive parameters and properties of astaxanthin esters manufactured by Fuji Chemical Industry Co. are summarized in Table 4. Astaxanthin esters produced as dark red viscous oil from *H. pluvialis* algae biomass employing a supercritical $CO_2$ or acetone extraction process is the subject of this GRAS determination. In the National Center for Biotechnology Information Entrez Taxonomy database, this alga is referred as: *Haematococcus pluvialis* J. von. Flotow 1844. Given the history of safe use, *H. pluvialis* alga is considered as nontoxigenic/nonpathogenic. The extraction method used for the preparation of astaxanthin esters from the algal biomass does not alter the chemical structure of astaxanthin. Both the supercritical $CO_2$ and the acetone extraction processes results in products that are essentially the same in their quantitative composition with the exception of slightly higher tocopherol and phospholipid values in the acetone extracted product. The acetone-extracted product is marketed in Japan and is approved as a food additive (*Haematococcus* algae color (No. 403), the List of Existing Food Additives, Ministry of Health and Welfare Notification No. 120, April 16, 1996). Additionally, Fuji Chemical Industry Co. also markets algal biomass. All these products are marketed under the trade name AstaREAL®.

**Table 4. General descriptive characteristics of astaxanthin esters**

| Parameter | Description |
|---|---|
| Source | *Haematococcus pluvialis* (microalgae) |
| Chemical name | Astaxanthin |
| Synonym, trade names | Astaxanthin esters; AstaREAL® |
| CAS Number | 472-61-7 (for astaxanthin) |
| EINECS Number | 207-451-4 (for astaxanthin) |
| Molecular weight | 596.84 (for astaxanthin) |
| Chemical formula | $C_{40}H_{52}O_4$ (for astaxanthin) |
| Physical state | Viscous oil |
| Solubility | Practically insoluble in water or hot water. Partially soluble in ethanol. Soluble in *n*-hexane, acetone, ether |
| Color | Dark red |
| Odor | Mild seaweed smell |
| Storage | Store in sealed bags at or less than 50°F. Not to exceed 86°F during transportation. Avoid light, heat and air |

### 1.3.1. Manufacturing Process

*H. pluvialis* is used as a starting material for the production of astaxanthin esters. The algae are grown in stainless steel photobioreactors, harvested and then dried under vigorous standards of hygiene. The details of the production process are presented in the process diagram below. Food grade antioxidant (Vitamin E) is added to the algal biomass in accordance with good manufacturing practices to stabilize astaxanthin. The algae cell walls are crushed in water and dried following addition of vitamin E. The resulting dry biomass is called AstaREAL® (formerly known as AstaCarox®). Astaxanthin esters are extracted with supercritical $CO_2$ or acetone from AstaREAL®. This process concentrates the lipid fraction including the carotenoids and is essentially the same as that used commonly in the food industry to defat a variety of products. Proprietary information on the cultivation medium shows that it is composed of mineral salts commonly used in growth media, necessary trace nutrients, and a GRAS, food grade acid for pH control. The extracted crude oil is astaxanthin esters. Processing aids used are food grade as specified in Food Chemicals Codex. Astaxanthin esters are manufactured in accordance with current Good Manufacturing Practices (cGMP).

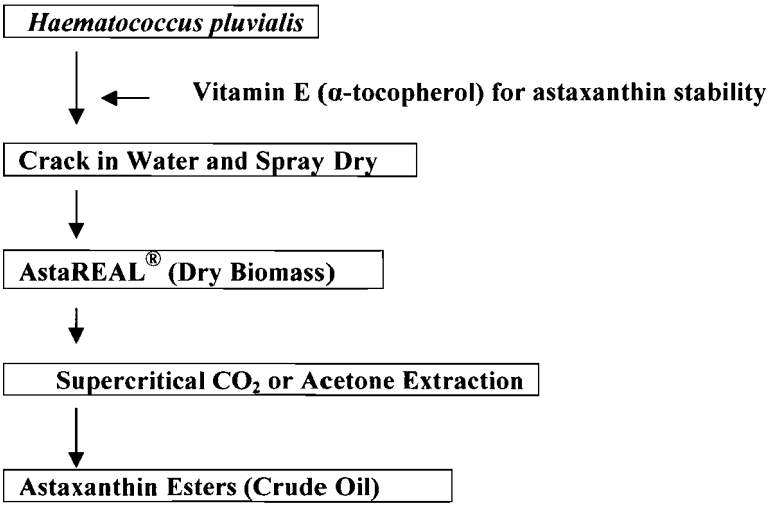


**Figure 2. Manufacturing process of astaxanthin esters**

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### 1.3.2. Identity and Specifications

Compositional analysis of astaxanthin esters from Fuji Chemical Industry Co. is presented in Table 1 (for individual batch analyses see Appendix I and Appendix II). In addition to compositional analysis, quantitative composition (from lot 1- Appendix I) was also performed at an independent laboratory and over 99% of the constituents were identified (Table 5). Astaxanthin in the extract is predominantly present in the esterified forms of commonly consumed fatty acids. For comparison purposes, compositional analysis from three lots of crude oil prepared using acetone extraction is summarized in Appendix II. The results from three non-consecutive batches indicate that the astaxanthin esters prepared using either the supercritical $CO_2$ extraction or acetone extraction methods are essentially similar (Appendix I and II).

**Table 5. Quantitative composition of astaxanthin esters from an independent laboratory***

| Chemical constituents | Composition (% w/w) | Remarks |
|---|---|---|
| Acylglycerols | 80.20 | Primarily triacylglycerol and traces of mono- and di- acylglycerol |
| Free fatty acids | 0.90 | |
| Carotenoids | | |
| Astaxanthin | 15.80 | Mainly monoesters and some diester and free form |
| Others | 0.20 | Lutein, adonirubin, β-carotene, catnthaxanthin |
| Subtotal | 16.00 | |
| Tocopherols | 2.15 | Mainly α-tocopherol |
| Phospholipids | 0.02 | As stereo-oleo-phospholipids |
| Moisture | 0.07 | |
| **Total (identified)** | **99.34** | |
| **Unidentified** | **0.66** | |

*Analyzed by Prof Dr. Kusumi of University of Tokushima, Japan; For this determination Lot No. 1 was submitted

Standard specifications of astaxanthin esters are summarized in Table 2. Analytical results from five lots (Appendix III & IV) demonstrate that astaxanthin esters are consistently manufactured to meet these specifications. The analytical method for determining astaxanthin content in its free form from AstaREAL® by Fuji Chemical Industry Co. is described in Appendix V. The typical fatty acid profile of astaxanthin esters is summarized in Table 3.

### 1.4. Technical Effects

Astaxanthin esters are intended for use in food as a nutrient for individuals who wish to increase their daily intake of astaxanthin. Astaxanthin is naturally present in several commonly consumed foods such as salmon, red fish, shrimp, krill and lobster. Although astaxanthin is present at a certain level in the diet, its supplementation to food as a nutrient provides an additional means of dietary intake for certain individuals. Vegetarians, vegans and certain others who do not consume significant quantities of foods containing astaxanthin as a natural constituent of the diet may wish to supplement their intake with added astaxanthin. Astaxanthin has been experimentally shown to protect cellular and mitochondrial membranes and ocular tissues against oxidative damage. As an antioxidant, astaxanthin is about ten times more powerful compared to other carotenoids.

While the use of astaxanthin in foods may also impart a color to products, the intended use of astaxanthin esters would fall outside the definition of a "color additive" for the following reasons: 1. The "non-apparent color" Exemption [21 CFR 70.3(f)]- The intended use levels are low enough that it dose not impart a significant color to the food products. Although astaxanthin esters have a natural red color, in many cases they will be added to food at levels so low that it will not affect the color of the food. Hence, for such types of uses, astaxanthin ester may not be regulated as "color additive." 2. "Unimportant color" Exemption [21 CFR 70.3(g)]- For some of the intended uses, astaxanthin ester, when added solely to provide nutritive value, would contribute a color in a manner that would conform to this exemption. 3. "Food Ingredient" Exemption [21 CFR 70.3(f)]- It is a food use and does not relate to any use of the ingredient as a color additive. The intended use of astaxanthin esters in certain specified foods is to provide consumers with a supplementary source of astaxanthin in their diet and does not relate to any use of the ingredient as a color additive [21 CFR 70.3(f)]. The above exemptions from the color additive definition will cover the intended uses of astaxanthin esters under the GRAS notification.

### 1.5. Current Uses

Astaxanthin is used extensively as a supplement in fish feed. It is approved by FDA as an additive (21 CFR 73.35) to salmonid fish feed (at up to 80 mg/kg feed) in order to obtain the desired pink to orange-red flesh color. In addition, other sources of astaxanthin such as *Haematococcus* algae meal (21 CFR 73.185) and Phaffia yeast (21 CFR 73.355), consisting of the dried cells of the alga *H. pluvialis* and yeast *Phaffia rhodozyma*, respectively, are also approved as additives to fish feed. The quantity of astaxanthin in finished feed, from *Haematococcus* algae meal or Phaffia yeast when used alone or in combination with other astaxanthin color additive sources listed in 21 CFR 73, shall not exceed 80 mg/kg of finished feed. Although the purpose of added astaxanthin in feeds for salmonid fishes is to impart coloration to fish flesh, it has been suggested that the carry-over of astaxanthin from farmed salmon into the human food chain should be beneficial to human health (Baker and Gunther, 2004).

Because of its potential health benefits (Higuera-Ciapara et al., 2006), astaxanthin is commonly marketed as a dietary supplement and is regulated under the Dietary Supplement Health and Education Act (DSHEA, 1994). Astaxanthin-containing products are also marketed for health benefits in Japan (FCN, 2001). Several *in vivo* and *in vitro* studies, including animal and human studies have shown that astaxanthin possesses a wide variety of biological activities including as an antioxidant (Esterbauer et al., 1987; Naguib, 2000; Dore, 2005), as an antitumor agent (Tanaka et al., 1995a; 1995b), for its anti-*Helicobacter pylori* effects (Wang et al., 2000), favorable effects on blood pressure (Hussein et al., 2005a; 2005b), as well as cardioprotective effect (Gross and Lockwood, 2004). Astaxanthin has also been shown to be a more potent antioxidant than other carotenoids and vitamin E (Miki et al., 1982; Terao, 1989). The antioxidant-related health benefits of astaxanthin have been purported to be due to its unique chemical structure.

### 1.6. Intake from Natural Presence in Food

Astaxanthin is a natural carotenoid pigment found in many animals and plants. It is responsible for the pink-to-red coloration of the flesh of various salmon species and

rainbow trout where it is found at concentration ranging from 3 to 40 mg/kg. In commercially farmed Atlantic salmon, astaxanthin levels range from 6 to 8 mg/kg flesh and it does not exceed 10 mg/kg (EFSA, 2005). In wild salmon species found on the Pacific coast of the US, Turujman et al. (1997) reported the presence of astaxanthin as high as 58 mg/kg in Sockeye salmon. The average astaxanthin levels in this study were reported as 13.8 mg/kg in Coho salmon and 40.4 mg/kg in Sockeye salmon. The main astaxanthin isomer identified in this study was the 3S,3′S stereoisomer identical to that found in *H. pluvialis*. In addition to salmon, astaxanthin is also found in crustacean species such as krill, shrimp, crab, lobster and crawfish. Thus, humans have ingested astaxanthin for centuries, with amounts depending on the importance of seafood in the diet .

The human intake of astaxanthin varies from country to country. The major food sources of astaxanthin are salmon and trout with limited exposure from krill or algae. The European Food Safety Authority (EFSA, 2005) reviewed the available information on human intake of astaxanthin. As the data on intake of trout and salmon were insufficient, three different models were used to estimate human intake. In the first method, based on salmon and trout production in Europe, the mean daily intake of astaxanthin was determined as 0.045 mg/person. In the second method, based on WHO GEMS figures of daily fish consumption in Europe, the daily intake of astaxanthin was estimated to range from 0.34 to 0.85 mg/day. The third estimate was based on individual country-based consumers-only intake of fish and seafood products, primarily derived from European Union reports on heavy metal and organotin compounds. Based on these data, for consumers with a high intake of fish and seafood, the estimated daily intake of astaxanthin ranged from 1.6 to 4.1 mg/day. Using the same calculations and figures reported for astaxanthin in Sockeye salmon, the resulting intake of astaxanthin was determined to range from 3.0 to 6.3 mg/day.

The US general population has a rather low intake of about 2-3 pounds/year of salmon with an estimated intake of 29 μg astaxanthin/person/day from wild and farmed salmon. The USDA database on foods commonly eaten in the US (Smiciklas-Wright et al., 2002) reported the 90$^{th}$ percentile estimated intake of fish (total finfish- includes fresh, frozen, canned, dried, and raw) for all individuals as 170 g/person/day. The average astaxanthin levels in Sockeye salmon from the Pacific coast of the US were reported as 40.4 mg/kg (Turujman et al., 1997). Assuming at the extreme, that all fish consumed is Sockeye salmon, the 90$^{th}$ percentile daily intake of astaxanthin would be 6.87 mg/person. This is an upper bound estimate and actual intakes are likely to be much lower.

Certain subpopulations, such as Alaskan natives, Greenland Eskimos and Japanese Fisherman have much higher intakes of salmon and seafood. While no epidemiological studies have been conducted to examine the health parameters and possible effects of high dietary intake of astaxanthin *per se* in these populations, available evidence points to health benefits including potentially reducing the risk of cardiovascular diseases from regular intake of salmon. Although the beneficial effects in these populations have been attributed to their omega-3 fatty acid intake from these dietary sources, they also consumed relatively high amounts of astaxanthin in the diet from wild salmon without any reported adverse effects.

## 1.7. Intended Use Levels and Food Categories

Fuji Chemical Industry Co. intends to use astaxanthin esters as a nutrient at levels of 0.5 mg astaxanthin/serving in Baked Goods, Beverages, Cereals, Chewing Gum, Coffee and Tea, Dairy Product Analogs, Frozen Dairy Desserts and Mixes, Hard Candy, Milk Products, Processed Fruits and Fruit Juices, Processed Vegetables and Vegetable Juices, and Soft Candy where not precluded by a Standard of Identity. Estimates of possible daily intake from the intended use levels of astaxanthin esters have also been determined using the CSFII 2003-04 (USDA, 2004) database [as determined by CanTox Health Sciences International (CanTox) see below] of the respective food product. Based on the maximum intended use levels of astaxanthin esters, the resulting daily intake of astaxanthin at the mean and 90$^{th}$ percentile level has been determined.

### 1.7.1. Estimated Daily Intake from the Intended Uses

Based on the exposure estimates prepared by CanTox (Appendix VI), approximately 97% of the total U.S. population was identified as consumers of astaxanthin (8030 actual users identified). Although, infants are included in the intake determinations, astaxanthin is not intended to be used in products such as baby foods or infant formula which are specifically marketed for use by infants. Consumption of the types of food categories intended for addition of astaxanthin by the total U.S. population resulted in estimated mean all-person and all-user intakes of astaxanthin of 3.1 mg/person/day (0.1 mg/kg body weight/day) and 3.2 mg/person/day (0.1 mg/kg body weight/day), respectively (Table 6). The 90$^{th}$ percentile all-person as well as all-user intake of astaxanthin from all intended food-uses of astaxanthin esters by the total population was 5.4 mg/person/day (0.1 mg/kg body weight/day). For details of the consumption analysis, please see Appendix VI. The FDA commonly uses the estimated daily intake for the 90$^{th}$ percentile consumer of a food ingredient as a measure of high chronic dietary intake. Hence, for the safety determinations, the resulting 90$^{th}$ percentile intake of astaxanthin (5.4 mg/person/day) from its intended uses in the above described food categories is considered. A summary of the estimated daily intake of astaxanthin from the intended food categories is presented in Table. 6.

**Table 6. Intake of astaxanthin (mg/person/day) (USDA , 2004)**

| Population Group | Age Group (Years) | % Users | Actual # of Total Users | All-Person Consumption (mg) | | All-Users Consumption (mg) | |
|---|---|---|---|---|---|---|---|
| | | | | Mean | 90$^{th}$ Percentile | Mean | 90$^{th}$ Percentile |
| Infant | 0 to 2 | 75.3 | 700 | 0.9 | 2.1 | 1.1 | 2.3 |
| Child | 3 to 11 | 100 | 1,287 | 2.4 | 3.9 | 2.4 | 3.9 |
| Female Teenager | 12 to 19 | 100 | 992 | 2.6 | 4.3 | 2.6 | 4.3 |
| Male Teenager | 12 to 19 | 100 | 999 | 3.4 | 5.4 | 3.4 | 5.4 |
| Female Adult | 20 and Up | 99.9 | 2,126 | 3.0 | 5.0 | 3.0 | 5.0 |
| Male Adult | 20 and Up | 99.8 | 1,926 | 3.8 | 6.3 | 3.8 | 6.3 |
| **Total Population** | **All Ages** | **97.1** | **8,030** | **3.1** | **5.4** | **3.2** | **5.4** |

### 1.8. Summary of Consumption

The estimated daily intake of astaxanthin from astaxanthin esters for the various food categories identified above was determined using CSFII 2003-04 (USDA, 2004). From these determinations, the high users only 90$^{th}$ percentile consumption value of astaxanthin for food uses in different products [Baked Goods, Beverages, Cereals, Chewing Gum, Coffee and Tea, Dairy Product Analogs, Frozen Dairy Desserts and Mixes, Hard Candy, Milk Products, Processed Fruits and Fruit Juices, Processed Vegetables and Vegetable Juices, and Soft Candy] was considered as the estimated daily intake resulting from the intended use of astaxanthin esters. Based on these considerations, the intended uses of astaxanthin esters will result in a daily 90$^{th}$ percentile exposure of astaxanthin of 5.4 mg/person (0.1 mg/kg body weight/day). Astaxanthin is naturally present in food and its intake from the diet ranges from 0.045 to 6.87 mg/day. Based on the available scientific information and as discussed below, the estimated daily intake, if ingested daily over a lifetime, is considered as safe.

## 2. TOXICOLOGY

In several experimental studies, astaxanthin either as an extract from natural sources, in its synthetic form, or as present in algal biomass has been evaluated for its safety. In the following section, studies of crude oil prepared using both types of extraction procedures (acetone extraction and a supercritical $CO_2$ extraction) resulting in astaxanthin esters are described. The compositional analysis of crude oil prepared using either procedure suggests that both manufacturing procedures result in essentially the same product (Appendix I and II) with the exception of slightly higher tocopherol and phospholipid values in the acetone extracted product. Hence, studies of the crude oil prepared by using either the acetone extraction or $CO_2$ extraction process are applicable to the safety determination of astaxanthin esters the subject of this safety determination.

The safety determination of astaxanthin esters is based on the totality of available evidence, particularly from human clinical observations, as well as animal experimental studies. The majority of the human studies of astaxanthin were designed to evaluate its efficacy as an antioxidant. These studies are reviewed as part of the safety evaluation, since in addition to efficacy, relevant safety endpoints were included. The assessment of efficacy studies is limited to a review of the results related to safety and tolerability. Relevant biological and toxicological studies on astaxanthin are included in the following section in the order of their importance to provide support for the conclusions reached in this determination.

### 2.1. Human Studies

The clinical database of astaxanthin intake includes over 25 clinical trials, 15 of which have been identified as double-blind, placebo-controlled trials. The objective of majority of these trials was to examine the effect of astaxanthin as an antioxidant. Although these investigations were designed to study the efficacy of astaxanthin, clinical observations reported whether any concomitant adverse effects occurred. In the clinical trials with astaxanthin, more than 600 subjects participated with treatment lasting from 2 weeks to 12 months. The doses used in these trials ranged from 2 to 40 mg/person/day. A summary of clinical trial designs, doses and adverse effects noted in these investigations is presented in Table 7.

In a double-blind, placebo-controlled randomized clinical trial, Spiller and Dewell (2003) investigated the safety of an astaxanthin extract (*H. pluvialis* algal extract). In this 8 week study, 35 healthy adults aged 35-69 years participated. All subjects were given three gel capsules/day, one at each meal. Of the 35 subjects, 19 took gel capsules with astaxanthin extract containing 2 mg of astaxanthin/capsule (6 mg/day), and 16 participants received the placebo gel capsules. The composition of gel capsules containing astaxanthin was as follows: 460 mg high-oleic safflower oil, 40 mg *H. pluvialis* algal extract, 35 μg lutein and 0.4 mg rosemary oil added to enhance stability. The placebo capsule contained 460 mg high-oleic safflower oil. No significant differences were detected between the treatment and placebo groups after 8 weeks of supplementation in blood cell counts, blood pressure and blood chemistry tests, including a comprehensive metabolic panel (a group of 14 specific tests), except for small changes in serum calcium, total protein and eosinophils. The increases in serum calcium, total protein and a decrease in eosinophil counts were minimal and considered to be of no clinical significance. The investigators concluded that 6 mg of astaxanthin/day from a *H. pluvialis* algal extract can be safely consumed.

In a double-blind, placebo-controlled, prospective trial, Kupcinskas et al. (2008) investigated the effects of astaxanthin in patients with functional dyspepsia and with or without *Helicobacter pylori* infection. The subjects were divided into three groups with 44 individuals in each group and were given either as placebo (containing dextrin), 16 mg, or 40 mg astaxanthin/day for 4 weeks. Although the objective of the study was to investigate the preventive effects of astaxanthin, no treatment-related adverse effects were noted. In a randomized controlled trial, 15 subjects were given daily astaxanthin (4 mg) along with vitamin C, vitamin E, zinc, copper, lutein and zeaxanthin for 12 months (Parisi et al., 2008). The investigators concluded that in nonadvanced age-related macular degeneration, supplementation with carotenoids and antioxidants was found to improve the function of the central retina. No adverse effects were noted in any of the subjects enrolled in the study during the entire period of treatment. In a 6 month double-blind, placebo-controlled trial, astaxanthin at a daily dose of 4 mg/day was given to 20 subjects and no adverse effects were noted as assessed by physical examinations and hemoglobin at the end of 3 and 6 months (Malmsten and Lignell, 2008).

Karppi et al. (2007) assigned 40 young healthy men (average age 24.4 years, average BMI 23.8) to receive daily supplements of astaxanthin (8 mg/day) or placebo for three-months in a randomized double-blind study. These investigators also studied absorption of astaxanthin (see also section 2.2.1.). The results supported the safety of astaxanthin as a dietary supplement, with no gastrointestinal tract distress reported, nor were there any changes in markers of inflammation or blood pressure. In an open label study, Satoh et al., (2009) evaluated clinical toxicity and efficacy of long-term administration of astaxanthin to healthy adult volunteers, by measuring biochemical and hematological blood parameters. In this study, 73, 38, and 16 healthy adult volunteers received 4, 8, and 20 mg astaxanthin (*H. pluvialis* extracts; Puresta®, Yamaha Motor Co., Ltd.), respectively, once daily for 4 weeks. No treatment-related adverse effects or changes in blood parameters were noted. In addition to these studies, several other double-blind, placebo-controlled, or open-label clinical trials (Table 7) did not reveal significant adverse effects from astaxanthin intake. Additionally, human pharmacokinetic

studies described separately (see also section 2.2.1.) also did not reveal any adverse effects of astaxanthin.

**Table 7. Findings from astaxanthin clinical trials**

| Reference; Study design; Substance used | Dose (mg/day); Duration | Number Subjects | Nature of trial | Findings |
|---|---|---|---|---|
| Andersen et al. (2007); Placebo-controlled; Biomass- AstaREAL® | 40 mg/day for 8 wk | 21 | Dyspepsia treatment | No adverse effects reported |
| Bloomer et al. (2005); DB-PC; Algal extract/astaxanthin | 4 mg/day for 3 wk | 10 | Endurance | No adverse effects reported |
| Comhaire et al. (2005); DB-PC; Biomass-AstaREAL® | 16 mg/day for 12 wk | 11 | Infertility | No adverse effects reported |
| Cicero et al. (2007); Single-blind; Astaxanthin | 6 mg/day for 4 wk | 20 | Dyslipidemias | No adverse events or impairments of liver transaminases or CPK |
| Iwamoto et al. (2000); Controlled; Krill extract/astaxanthin | 1.8, 3.6,14.4, 21.6 mg/day for 2 wk | 5, 5, 3, 5, respectively | LDL Oxidation | No adverse effects reported |
| Iwasaki and Tawara (2006); DB-PC, crossover; AstaREAL® | 6 mg/day 2 wk | 10 | Eye function | No adverse effects reported |
| Karppi et al. (2007); DB-PC; Biomass-AstaREAL® | 8 mg/day for 12 wk | 20 | Safety | Well tolerated |
| Kupcinskas et al. (2008); DB-PC prospective; Biomass- AstaREAL® | 0, 16, 40 mg/day in algal meal for 4 wk | 44, 43, 44, respectively | Dyspepsia treatment | No treatment-related adverse effects were noted |
| Kuge and Silver (1999); Astaxanthin extract; Placebo-controlled | 3.85 or 19.25 mg/day for 4 wk | 33 | Safety | No adverse effects noted; blood chemistry analyses and urinalysis were conducted four times through the trial |
| Lignell et al. (1999); Open label; Biomass-AstaREAL® | 40 mg/day 3 wk | 10 | Dyspepsia; safety | No adverse effects reported |
| Malmsten and Lignell (2008); DB-PC; Biomass-AstaREAL® | 4 mg/day for 24 wk | 20 | Endurance; safety | No adverse effects reported. No changes in hemoglobin at 3 and 6 months |
| Miyawaki et al. (2005; 2008); single blind-PC; Extract-AstaREAL® | 6 mg/day for 10 days | 10 | Safety; blood flow | No adverse effects reported |
| Nagaki et al. (2002); DB-PC; Extract-AstaREAL® | 5 mg/day for 4 wk | 13 | Eye function | No adverse effects noted |
| Nagaki et al. (2005); DB-PC; Extract-AstaREAL® | 6 mg/day for 4 wk | 25 | Retinal blood flow; safety | No treatment-related adverse effects noted. No treatment-related effects on hematology, serum chemistry |
| Nagaki et al. (2006); DB- | 6 mg/day for 4 wk | 31 | Eye | No treatment-related adverse |

| Reference; Study design; Substance used | Dose (mg/day); Duration | Number Subjects | Nature of trial | Findings |
|---|---|---|---|---|
| PC; Extract-AstaREAL® | | | function; safety | effects noted. No treatment-related effects on hematology, serum chemistry |
| Nagata et al. (2006); DB-PC-crossover; Extract-AstaREAL® | 5 mg/day for 2 wk | 19 | Exercise endurance | No adverse reaction noted. |
| Nakamura et al. (2004); DB-PC; Extract-AstaREAL® | 2, 4, 12 mg/day for 4 wk | 39 | Eye function; safety | No adverse effects noted. |
| Nitta et al. (2005); DB-PC; Extract-AstaREAL® | 6, 12 mg/day for 4 wk | 20 | Eye function; safety | No adverse effects noted. No effects on hematology, serum chemistry |
| Ohgami et al. (2005); Open label; Extract-AstaREAL® | 30 mg/day for 4 wk | 10 | Safety/Toxicity | No adverse effects noted. No effects on hematology, serum chemistry, urine analysis during treatment (2 and 4 wks) and after the treatment (2 and 4 wks) |
| Parisi et al. (2008); Randomized- controlled; astaxanthin | 4 mg/day for 52 wk | 15 | Eye function | No adverse effects noted |
| Satoh et al., (2009); Open label; Astaxanthin-rich *H. pluvialis* oil | 4, 8, 20 mg/day for 4 wk | 73; 38; 16, respectively | Safety/Toxicity | No adverse effects noted. No effects on hematology, serum chemistry, urine analysis |
| Satoh et al., (2009); Open label; Astaxanthin-rich *H. pluvialis* oil | 12 mg/day for 12 wk | 10 | Safety; Forgetfulness | No adverse effects reported |
| Sawaki et al. (2002); DB-PC; Extract-AstaREAL® | 6 mg/day for 4 wk; 6 mg/day for 4 weeks | 9; 8 | Endurance; Eye function; safety | No adverse effects reported. No effects on hematology, serum chemistry |
| Shiratori et al. (2005); DB-PC; Extract-AstaREAL® | 6 mg/day for 4 wk | 20 | Eye function | No adverse effects noted |
| Spiller and Dewell (2003); DB-PC; Algal extract/astaxanthin | 6 mg/day for 8 wk | 19 | Safety | No effects on clinical chemistry, blood pressure and blood counts; increased serum calcium, protein and eosinophil count, not considered clinically significant |
| Takahashi and Kajita (2005); Open label; Extract-AstaREAL® | 6 mg/day for 2 wk | 10 | Eye function | No adverse effects reported |
| Tsukahara et al. (2005); Open label; Extract-AstaREAL® | 6 mg/day for 12 wk | 15 | Safety | No adverse effects noted. No effects on hematology, serum chemistry, urine analysis |
| Tsukahara et al. (2008); Open label; Extract-AstaREAL® | 6 mg/day for 4 wk | 13 | Blood flow; safety | No adverse effects reported |

| Reference; Study design; Substance used | Dose (mg/day); Duration | Number Subjects | Nature of trial | Findings |
|---|---|---|---|---|
| DB-PC = double-blind placebo-controlled; wks = weeks | | | | |

In summary, the results from 15 placebo-controlled, double-blind clinical trials and other human studies, employing doses of astaxanthin from 2 to 40 mg/day, from 4 weeks up to 12 months, did not reveal any significant adverse effects. The majority of the side effects observed were minor and transient and the frequency of adverse effects was not related to the duration or intake of astaxanthin. In some of these trials, hematology and clinical chemistry parameters were also studied and no treatment-related changes in these parameters were noted. No drug interactions or contraindications were reported. Because double-blind, placebo-controlled studies are considered the least likely to result in bias; the clinical studies of astaxanthin provide an opportunity to assess the safety and "tolerability" in a fairly diverse population. Collectively, these studies are of reasonably good quality and consistency and demonstrated that astaxanthin did not cause adverse effects and was well tolerated. In majority of the human clinical studies (20 out of 28), the subject of this GRAS determination AstaREAL® was used.

## 2.2. Absorption, Distribution, Metabolism and Elimination (ADME)

### 2.2.1. Human ADME Studies

In a double-blind placebo-controlled trial, Karppi et al. (2007) reported that in humans (n=20) consuming 8 mg astaxanthin/day for 12 weeks, plasma astaxanthin levels were significantly elevated to 0.032 pmol/L as compared to the placebo group. The results of this study demonstrate that orally administered astaxanthin is absorbed and bioavailable in humans. In another study, Osterlie et al. (2000) investigated the pharmacokinetics of synthetic astaxanthin isomers E/Z and R/S in 3 middle-aged male volunteers (37-43 years) following a single meal ingestion containing 100 mg astaxanthin. The astaxanthin source consisted of 74% all-E-, 9% 9Z-, 17% 13Z-astaxanthin (3R,3'R-, 3R,3'S; *meso*-, and 3S,3'S-astaxanthin in a 1:2:1 ratio). Maximum levels of astaxanthin (1.3 mg/L) were reached at 6.7 hours following administration, and the plasma astaxanthin elimination half-life was 21 hours. Astaxanthin was present primarily in very low-density lipoproteins containing chylomicrons (VLDL/CM; 36-64% of total astaxanthin), whereas low-density lipoprotein (LDL) and high-density lipoprotein (HDL) contained 29% and 24% of total astaxanthin, respectively. These results suggest that following ingestion, astaxanthin was found in all lipoprotein fractions.

Coral-Hinostroza et al. (2004) also investigated the pharmacokinetics of astaxanthin in three human volunteers. This study was conducted on two separate occasions: volunteers were first given a single meal containing a 10 mg dose equivalent of astaxanthin from astaxanthin diesters and subsequently a dose of 100 mg astaxanthin equivalents after 4 weeks. After administration of 100 mg astaxanthin, plasma astaxanthin concentrations reached a maximum ($C_{max}$) of 0.28 mg/L at 11.5 hours and declined slowly thereafter. At 76 hours, ~11% of $C_{max}$ (0.08 mg astaxanthin/L) was still present in the plasma. Following administration of 10 mg astaxanthin, a $C_{max}$ of 0.08 mg/L was obtained and a small amount of unesterified astaxanthin was detected only after 6 and 7.5 hours. These observations differed from those described by Osterlie et al.

(2000), in that the pharmacokinetics was slower (peak plasma astaxanthin concentration achieved at 11.5 hours, plasma elimination half-life was highly variable >52 hours). In this study, no esterified astaxanthin was detected in the plasma, suggesting that hydrolysis of the esterified astaxanthin prior to absorption may be the rate-limiting step. The results from both the Coral-Hinostroza et al. (2004) and Osterlie et al. (2000) studies suggest that the uptake of astaxanthin after oral administration of the corresponding diesters in men was lower compared to when unesterified astaxanthin was administered. In the Osterlie et al. (2000) study, synthetic, water soluble unesterified astaxanthin was used while in Coral-Hinostroza et al. (2004) astaxanthin diesters were used. These differences may be responsible for the differential uptake. However, Mercke Odeberg et al. (2003) reported enhanced oral bioavailability of esterified astaxanthin administered as *Haematococcus* algae meal in human volunteers by the incorporation of the astaxanthin into lipid-based formulations. These observations suggest that dissolution from the matrix and/or incorporation into mixed micelles may also limit the rate of absorption.

Absorption of carotenoids from the diet occurs by passive diffusion into the intestinal epithelium, a process that requires small amounts of fat. In their bioavailability study, Mercke Odeberg et al. (2003) investigated the pharmacokinetics of astaxanthin. In this open-label parallel study, healthy male volunteers (n=32) received a single dose of 40 mg astaxanthin (extract from *H. pluvialis*), as lipid-based formulations or as a commercially available food supplement. Blood samples were collected and pharmacokinetic parameters were calculated to evaluate the extent and rate of absorption from each formulation. The elimination half-life of astaxanthin was 15.9 hours, 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.

Kistler et al. (2002) investigated the metabolism of astaxanthin as well as its capacity to induce cytochrome P450 genes in primary cultures of human hepatocytes. This *in vitro* metabolic study of astaxanthin in primary human hepatocytes identified the formation of 3-hydroxy-4-oxo-β-ionol and 3-hydroxy-4-oxo-β-ionone as the main free metabolites. The same compounds and their reduction products were also present as glucuronides. In cultured hepatocytes, astaxanthin did not affect cytochrome P450 enzymes CYP1A and CYP2C families, while it induced CYP3A4 as well as of CYP2B6. Also, astaxanthin at a concentration of 3.75 μM was found to induce CYP3A4 in cultured hepatocytes, without affecting other CYP genes. However, at a lower concentration of astaxanthin (0.375 μM), no induction of CYP3A4 was noted. In clinical trials where patients have been on 40 mg astaxanthin/day for a period of 4 weeks (Table 7) the plasma level of astaxanthin does not exceed 125 μg/L plasma (Kupcinskas et al., 2008). The dose of astaxanthin (40 mg/day) used in the Kupcinskas et al. (2008) study was approximately 10 times higher than the recommended dose of astaxanthin as a dietary supplement. The plasma level of 125 μg/L corresponds to about 0.2 μM astaxanthin. In the Kistler et al. (2002) study, no induction of CYP3A4 was noted at concentrations of 0.375 μM. These

observations suggest that at the intended use levels (5 mg/day), astaxanthin is not expected to affect CYP enzymes or metabolism of drugs.

A limited *in vivo* experiment in two subjects conducted by Kistler et al. (2002) revealed the presence of four metabolites (also noted in hepatocyte culture) in the plasma taken from these volunteers 24 hours after oral administration of 100 mg astaxanthin (8% gelatin formulated beadlets). These investigators showed that the C9,C9′ cleavage of the astaxanthin molecule and reduction of the polyenic 7,8-double bond are common to the human and the rat, whereas the reduction of β-ionone to β-ionol is specific to the human. The lack of autoinduction of astaxanthin metabolism in human hepatocytes suggests that neither CYP3A4 nor CYP2B6 contribute to the formation of metabolites. These investigators concluded that metabolism of astaxanthin and its CYP-inducing capacity are different in humans and in rats.

In a randomized, double-blind trial, Rufer et al. (2008) investigated the bioavailability and isomer distribution of astaxanthin in human plasma after ingestion of wild (Oncorhynchus spp.) and aqua cultured (*Salmo salar*) salmon. In this study, 28 men consumed 250 g of wild or aqua cultured salmon daily for 4 weeks with each providing 5 μg astaxanthin/g salmon flesh (1.25 mg astaxanthin/person/day). After 6 days of wild and aqua cultured salmon consumption, plasma astaxanthin concentrations reached a plateau of 39 nmol/L and 52 nmol/L, respectively. At days 3, 6, 10 and 14 - but not at day 28 - the astaxanthin concentrations in human plasma were significantly greater after ingestion of aqua cultured salmon. After consumption of wild salmon, the (3S,3'S) isomer predominated in plasma (80%), whereas after intake of aqua cultured salmon the *meso* form (3R,3'S) prevailed (48%). The astaxanthin isomer pattern in human plasma resembles that of the ingested salmon. Consumption of both wild and aqua cultured salmon for 28 days resulted in the relative proportion of the (3S,3'S) isomer slightly higher and the (3R,3'R) form lower in human plasma compared with the isomer distribution in salmon flesh. The results of this study indicate that a selective process of isomer absorption may be responsible for the observed differences in the relative proportions of the (3S,3'S) and (3R,3'R) isomers in human plasma compared with salmon flesh. In another study reported in German language with an English abstract, Elmadfa and Majchrzak (1999) reported that following ingestion of 500 g of salmon meal containing 2.48 μmol astaxanthin by five volunteers, no astaxanthin was detected in plasma while 64% of astaxanthin was excreted in feces within the following 3 days. This study suggests that majority of the ingested astaxanthin is excreted in feces.

In summary, human studies indicate that as a fat-soluble compound, astaxanthin generally follows the same digestive and absorptive pathways as dietary fat. Following oral administration, astaxanthin appeared in the plasma. Orally administered astaxanthin is incorporated into chylomicrons, and distributed between VLDL, HDL and LDL fractions in the circulation. *In vivo* experiments revealed the presence of four astaxanthin metabolites. The available evidence suggests that oral intake of esterified astaxanthin and free astaxanthin results in only free astaxanthin being present in the plasma. However, the rate of accumulation in the plasma is slower with esterified astaxanthin as hydrolysis of the esterified astaxanthin must first take place prior to absorption. Lipid-based formulations containing esterified astaxanthin revealed enhanced bioavailability of astaxanthin. The available evidence indicates that at the levels intended for human

consumption, astaxanthin is unlikely to affect cytochrome P450 levels (please also see Section 2.3). Esterified astaxanthin must first be hydrolyzed in the stomach to free astaxanthin, as it is the free form that is absorbed and enters the circulation. The results from studies with both unesterified astaxanthin and esterified astaxanthin are equally valid for purposes of assessing the safety of astaxanthin esters.

### 2.2.2. Animal ADME Studies

Kurihara et al. (2002) reported that orally administered free astaxanthin (100 mg/kg/day) to C57BL/6 mice appeared in the plasma and liver as measured at 3, 5 and 7 hours. In other studies in mice, orally administered esterified astaxanthin, as a synthetically produced disodium disuccinate diester of astaxanthin studied for over 72 hours after single- and multiple-dose regimens (Showalter et al., 2004) or as an extract of *Haematococcus* algae (Aoi et al. 2003), also appeared as free astaxanthin in serum, liver, heart, gastrocnemius muscle, and to a lesser extent in the brain. The free astaxanthin appeared more rapidly in the liver compared to that in the serum (Showalter et al., 2004). In the study by Aoi et al. (2003) mice were fed a diet containing 0.02% astaxanthin for three weeks and astaxanthin was found in liver, heart, gastrocnemius. The pattern of accumulation of free and esterified astaxanthin in mice appears different.

Wolz et al. (1999) studied the *in vitro* metabolism of astaxanthin in primary cultures of hepatocytes obtained from rats fed a diet containing astaxanthin (300 mg/kg body weight) for four days. The metabolite found was (*rac*)-3-hydroxy-4-oxo-β-ionone, which was conjugated mainly into glucuronides. These investigators also noted that astaxanthin induces xenobiotic-metabolizing enzymes in rat liver *in vivo*. However, no differences were noted in the metabolism of astaxanthin in cultured hepatocytes from rats pretreated with astaxanthin. Neither liver microsomes from rats pretreated with astaxanthin nor control rats metabolized astaxanthin. The results of this study indicate that the cytochrome P450 enzymes were not involved in the metabolism of astaxanthin in rat hepatocytes. These investigators concluded that astaxanthin was metabolized in primary cultures of rat hepatocytes into (*rac*)-3-hydroxy-4-oxo-β-ionone and its reduced form (*rac*)-3-hydroxy-4-oxo-7,8-dihydro-β-ionone independent of the xenobiotic-metabolizing enzymes induced by astaxanthin.

In three separate abstracts presented at a conference, and as part of a Patent application, Chew et al. (2004a; 2004b; 2004c; 2004d) reported absorption as well as potential effects of astaxanthin extract from *Haematococcus* algae in dogs and cats. As these studies were published in abstract form, limited information was available. In the first experiment, female Beagle dogs were supplemented orally via diet with up to 40 mg of astaxanthin extract daily for 16 days (Chew et al., 2004b). Similarly, female domestic short-hair cats were given up to 10 mg of astaxanthin extract for 15 days. In both dogs and cats, astaxanthin appeared in plasma in a time and dose-dependent fashion. Astaxanthin was transported mainly by HDL and was incorporated into all subcellular organelles of blood leukocytes. In the second study, female Beagle dogs were orally administered up to 40 mg of astaxanthin extract for 16 weeks (Chew et al., 2004c). A dose-related increase in plasma astaxanthin was noted. In the third study, female domestic short-hair cats were orally up to 10 mg of astaxanthin daily for 12 weeks (Chew et al., 2004d). Astaxanthin was absorbed into the plasma in a dose-related fashion. In all these studies, no differences in body weight or blood hematology were noted between

astaxanthin-supplemented and control animals. Unfortunately, the details of these studies were not available for independent review.

In a subchronic feeding study (see section 2.5.) by Stewart et al. (2008), plasma concentrations of astaxanthin were determined on days 2, 8, 31 and 91 in rats fed diets containing 20% biomass (17076 mg/kg body weight/day, or 557 mg astaxanthin/kg body weight/day). Plasma concentrations of astaxanthin during the early period of exposure (i.e. at the end of 2 or 8 days) were similar to that noted at the end of 31 or 91 days suggesting that there is no accumulation over time. Astaxanthin concentrations ranged from 39.5 to 190.2 ng/ml. In another study, Gradelet et al. (1996) also reported high variability of plasma astaxanthin in rats fed astaxanthin in the diet at levels up to 300 mg/kg diet. The results of a tissue distribution study of astaxanthin in rats following dietary exposure to 0.3, 1, and 3% of astaxanthin in feed for 7 and 14 days (Petri and Lundebye, 2007) provide supportive data for the rapid elimination or catabolism of astaxanthin without long-term storage of astaxanthin in tissues. Taken together, these observations from different studies support the conclusion that duration of exposure to astaxanthin is not related to the accumulation of astaxanthin as reflected by its plasma concentration over time.

In summary, orally administered astaxanthin is absorbed but its bioavailability is low. Astaxanthin is metabolized to (*rac*)-3-hydroxy-4-oxo-β-ionone, which is conjugated mainly into glucuronides before elimination. The available evidence indicates that astaxanthin is unlikely to be metabolized by the microsomal xenobiotic-metabolizing enzymes. Short-term and subchronic animal studies suggest that astaxanthin is rapidly eliminated or metabolized, the length of exposure to astaxanthin is not related to the plasma concentration and astaxanthin is unlikely to be accumulated in the body.

### 2.3. Astaxanthin and Cytochrome P450

Jewell and O'Brien (1999) fed groups of male Wistar rats a diet containing 300 mg carotenoid/kg (β-carotene, bixin, lycopene, lutein, canthaxanthin or astaxanthin) for 16 days and studied changes in xenobiotic enzymes in liver, lung, kidney and small intestine. Cytochrome P450 activity was assessed using the substrates ethoxyresorufin for CYP1A1, methoxyresorufin for CYP1A2, pentoxyresorufin for CYP2B1/2 and benzyloxyresorufin for CYP types 1A1/2, 2B1/2 and 3A. Glutathione-S-transferase and reduced glutathione status were also assessed. Carotenoid uptake by the tissues was also determined. Compared to the control group, bixin, canthaxanthin and astaxanthin-treated groups revealed a significant increase in liver, lung and kidney CYP1A1. These three carotenoids also significantly induced CYP1A2 activity in liver and lung, while only canthaxanthin and astaxanthin increased activity in kidney. CYP2B1/2 and CYP1A1/2, 2B1/2, 3A were induced in liver to a lesser degree by canthaxanthin, astaxanthin and bixin. Marker enzyme of CYP1A1/2, 2B1/2, 3A in lung was significantly decreased by all carotenoids. Activities of any of the measured enzymes in the small intestine were undetectable in all carotenoid treated groups. Glutathione status was unaffected by any of the treatments. The results of this study indicate that astaxanthin induces xenobiotic metabolizing enzymes at a very high exposure level.

In another study, Astorg et al. (1997) compared the effects of carotenoids (β-carotene, canthaxanthin, astaxanthin, β-apo8'-carotenal) on mouse liver xenobiotic

enzymes. In this study male Swiss mice were fed diets containing β-carotene, β-apo-8'-carotenal, canthaxanthin, or astaxanthin (300 mg/kg diet) or treated with 3-methylcholanthrene (3 times at 50 mg/kg intraperitoneal) for 15 days. In earlier studies, these investigators reported induction of CYP1A following administration of canthaxanthin, astaxanthin, and β-apo8'-carotenal in rat liver and by 3-methylcholanthere in Swiss mice. In contrast to the reported finding from rat liver, only canthaxanthin showed some inducing effect on mice liver CYP1A- dependent activities with a much weaker intensity than 3-methylcholanthere. Interestingly, astaxanthin and β-apo-8'-carotenal showed no inducing activities. These authors noted that the fact that carotenoids have different effects on cytochrome P450 induction in Wistar rats and Swiss mice was not due to a lack of responsivity of Swiss mice to CYP1A inducers, as 3-methylcholanthere showed approximately same potency as an inducer in mice as in rats.

Another study reported that canthaxanthin and astaxanthin (100 mg/kg diet for three weeks) do not induce liver or kidney xenobiotic metabolizing enzymes in rainbow trout (Page and Davies, 2002). In this study, a range of cytochrome P450 markers including ethoxyresorufin o-deethylase (CYP1A1), methoxyresorufin o-demethylase (CYP1A2), pentoxyresorufin o-dealkylase (CYP2B1/2) and benzyloxyresorufin o-dealkylase (CYP1A1/2, 2B1/2 and 3A) were investigated. These findings are in contrast to the above described results from rat studies.

Kistler et al. (2002) investigated the capacity of astaxanthin to induce cytochrome P450 genes using human hepatocytes grown in primary cultures in an *in vitro* model. In this study, the liver samples used for the preparation of primary culture were obtained from patients who underwent a partial hepatectomy for the resection of liver metastases of various origins. The results of this study indicated that exposure of astaxanthin to hepatocytes for 96 hours at concentration of 3.75 μM induces CYP3A4 and CYP2B6 but not CYP1A1 and CYP1A2. However, as discussed above (Section 2.2.1.) at lower concentrations astaxanthin (0.375 μM) did not induce CYP3A4 and CYP2B6 genes. The results of this investigation demonstrate that astaxanthin does not induce CYP1A1 and CYP1A2 genes as well as CYP3A4 and CYP2B6 at levels of astaxanthin to which humans are likely to be exposed. As discussed earlier, in clinical trials where patients have been on 40 mg astaxanthin/day for 4 weeks, the plasma level of astaxanthin did not exceed 125 μg/L (Kupcinskas et al., 2008). In this case, 40 mg astaxanthin/day is 8-fold higher than the resulting 90$^{th}$ percentile intake of astaxanthin from its intended food uses. The plasma level of 125 μg/L corresponds to about 0.2 μM astaxanthin. At this concentration of astaxanthin, there was no induction of CYP3A4 and CYP2B6 genes.

Gradelet et al. (1997) demonstrated that dietary intake of carotenoids (canthaxanthin, astaxanthin, β-apo-8'-carotenal) which are inducers of ethoxyresorufin-O-deethylation (EROD) and methoxyresorufin-O-demethylation (MROD) reduced the carcinogenicity of aflatoxin B1, diverted aflatoxin B1 metabolism into the less genotoxic aflatoxin M1 form and reduced aflatoxin B1-induced single-strand DNA breaks in rat liver. However, induction of P450 enzymes can be potentially deleterious in certain situations, for example if induction enhances the conversion of procarcinogens to active metabolites. Available evidence suggests that astaxanthin in humans is unlikely to affect cytochrome P450 enzymes or drug metabolism. This is supported by the following evidence: First the species differences in P450 induction. The relatively poor correlation

between findings in rats and mice in relation to factors influencing xenobiotic metabolizing enzyme activities suggest the species differences. Human studies also suggest that metabolism of astaxanthin and its CYP-inducing capacity are different in humans than what is observed in rats. Similarly, feeding of a diet containing astaxanthin to rainbow trout did not induce liver or kidney cytochrome P450 enzymes. Secondly, the doses at which induction in cytochrome P450 enzymes was noted in rat studies were significantly higher compared to the doses to which humans are likely to be exposed. Thirdly, the available metabolism studies suggest that astaxanthin is unlikely to bioaccumulate. Finally, studies using human hepatocytes did not reveal P450 inducing effects of astaxanthin at likely human exposure levels. Thus the available evidence suggests that astaxanthin is unlikely to affect cytochrome P450 enzymes in humans.

### 2.4. Acute and Subchronic Studies of Extract or Synthetic Astaxanthin

In an acute oral toxicity study, administration of an astaxanthin-rich (acetone) extract of *H. pluvialis* to male and female Sprague-Dawley rats at a dose of 2000 mg/kg body weight did not result in any mortality (Takahashi et al., 2005). In a safety summary report submitted as part of a "color additive petition" on astaxanthin, the acute effects of astaxanthin in rats were determined following daily oral administration of up to 2000 mg astaxanthin/kg body weight for 10 consecutive days (Roche, 1987). Astaxanthin administration did not result in any mortality or symptoms of toxicity. The $LD_{50}$ of astaxanthin in this study was reported as greater than 2000 mg/kg body weight.

In a subchronic study, Takahashi et al. (2005) reported that daily oral (gavage) administration of an astaxanthin-rich (acetone) extract of *H. pluvialis* resulting in a daily intake of up to 50 mg astaxanthin/kg/day to Sprague-Dawley rats for 90 days did not reveal treatment-related adverse effects. The extract tested in this study is marketed as AstaREAL® Oil 50F (prepared using the acetone extraction procedure, the subject of this GRAS determination), which contains 5% astaxanthin in the free form. In this study, four groups of Crj:CD(SD)IGS rats (10/group/sex) were gavage administered daily with the extract at a dose of 0, 37, 185 and 926 mg/kg for 90 days. The dose of the extract resulted in intakes of astaxanthin equivalent to 0, 2, 10 and 50 mg/kg/day. No lethality was noted during the course of the study. Administration of the astaxanthin-rich (acetone) extract did not affect body weight gain or feed consumption. Except for an increase in prothrombin time (PT) and activated partial thromboplastin time (APTT) in male rats at the highest dose level (926 mg/kg) of the astaxanthin-rich extract administration, hematological examinations did not reveal any significant changes. The increases noted in PT and APTT were within historical control values for this species and sex in this laboratory and while not observed in similarly treated female rats the investigators did not consider these findings to be of any biological significance. No effects from treatment with astaxanthin-rich extract were observed in serum clinical chemistry parameters, urinalysis, organ weights or in histopathological examinations of major tissues (heart, lung, kidney, spleen, liver, stomach, and duodenum). In this study, an orange color of stools, proventriculus mucous membrane, and caecal content was observed following exposure to an astaxanthin-rich extract. Histopathological observations of gastric mucosa did not reveal any adverse findings. These color findings were thought to be the result of adhesion of the test substance pigment to the gastric mucosa. Based on the results of this study, the no-observed adverse-effect-level (NOAEL) of the astaxanthin-rich extract was

determined as 925.9 mg/kg/day (50 mg astaxanthin/kg/day), the highest dose tested. The overall specifications of AstaREAL® Oil 50F used in the Takahashi et al. (2005) study are essentially the same as the extract prepared using the supercritical extraction method.

In another 13-week oral toxicity study, the effects of dietary administration of phaffia color, which contains astaxanthin, to F344 rats was investigated (Onodera et al., 1997). Five groups of rats (10/group/sex) were fed CRF-1 powder diet containing 0, 0.2, 0.6, 1.7 and 5.0% phaffia color for 90 days. No lethality was noted during the feeding period. Feeding of phaffia color had no effect on body weight gain, hematological or biochemical parameters. Similarly, no treatment-related histopathological changes were noted. The investigators concluded that feeding of 5% phaffia color in the diet for 13 weeks was without any toxicological consequences in rats. Assuming an astaxanthin content of the phaffia color equal to the minimum level approved by the FDA for the product of 80 mg/kg, 5% phaffia color in the diet provided a daily exposure of approximately 20 mg astaxanthin/kg/day. The astaxanthin isomer found in phaffia is primarily (>98%) the 3R,3′R form, while in *H. pluvialis* it is exclusively (100%) 3S,3′S.

Ikeuchi et al. (2007) investigated the effects of astaxanthin supplementation in obese mice fed a high fat diet. In this study, female ddY mice (4 week old) were divided in five groups. One group received normal diet while other groups received either the high fat diet or high fat diet plus astaxanthin (AstaREAL®). The mice received either vehicle (olive oil) or astaxanthin in doses of 1.2, 6, and 30 mg/kg body weight/day via oral gavage for 60 days. Body weights and feed intake were recorded during the course of study and at the end of 60 days all animals were euthanized and blood samples were collected. The results of this study revealed that increases in body weight and adipose tissue were prevented in mice fed a high fat diet plus astaxanthin without changing the energy intake. At the end of experiment, astaxanthin inhibition of body weight gain and adipose tissue weights was dose-dependent. Astaxanthin reduced liver weight, liver triglyceride, plasma triglyceride, and total cholesterol. The results of this study indicate that astaxanthin administration does not affect energy intake.

In a color additive petition (Roche, 1987), astaxanthin in the form of gelatin beadlets in the diet at concentrations of 0 (placebo beadlets), 6.25, 12.5, and 25.0% was fed to rats for 13 weeks. The corresponding intake of astaxanthin was reported as approximately 0, 310, 620, and 1240 mg astaxanthin/kg body weight/day. No mortality due to astaxanthin treatment was noted. Compared to control rats, astaxanthin administration did not affect body weights. Feces of astaxanthin-fed animals were reddish in color and at autopsy, a yellow pigmentation of the adipose tissue was noted in astaxanthin-treated rats. There were no ophthalmoscopic findings related to astaxanthin. Decreases in organ weights (kidney, ovary, adrenals, uterus, and spleen) were noted in the mid- and high-dose treated groups. Except for decreased total serum protein levels (males in mid-and high-dose groups), and sporadic increases in liver enzymes of several animals, the hematology and blood chemistry parameters were within or close to normal historical ranges. Plasma cholesterol was higher in all treated groups but values were still within normal limits. The petitioner concluded that administration of astaxanthin at dose levels of 310, 620, and 1240 mg astaxanthin/kg body weight/day for 13 weeks was well tolerated and there was no apparent toxicity attributable to astaxanthin exposure.

In the same petition (Roche, 1987), a tolerance study in dogs was performed. Four groups of dogs (3/sex/group) were administered astaxanthin at concentrations of 0 (placebo), 2.5, 5.0, and 10.0% w/w astaxanthin beadlets in their feed. For these groups, the average astaxanthin intake for the 13 week period was reported as 0, 41, 76, and 162 mg/kg body weight/day, respectively. The test article was well tolerated and did not cause any adverse effects or show any evidence of systemic toxicity with regard to general physical health condition, body weight gains, behavior, hematological and clinical chemistry parameters, opthalmoscopy, and gross and histopathological changes of the organs. The results of this study did not reveal any adverse effects of astaxanthin in dogs.

In addition to the specifically designed toxicity studies described above, some studies reported observations on specific parameters, including cancer prevention in experimental animals exposed to astaxanthin (Uchiyama et al., 2002; Gradelet et al., 1997; Gradelet et al., 1998; Jewell and O'Brien, 1999; Kang et al., 2001; Jyonouchi et al., 2000; Tanaka et al., 1994). Although these studies were not designed to investigate the toxicity of astaxanthin, no adverse effects were noted. Tanaka et al. (1995a) investigated the chemopreventive effects of astaxanthin in rat oral mucosal tissues. In this study, 4-nitroquinoline 1-oxide exposed male F344 rats were fed diets containing 100 mg astaxanthin/kg for 32 weeks. One group of rats (n=12) was fed a diet containing 100 mg astaxanthin/kg alone for 32 weeks. The resulting astaxanthin intake was estimated to be 50 mg/kg body weight/day. Compared to rats in the control group, mean body, liver and percentage liver weights in the astaxanthin-only fed group were significantly lower. Dietary administration of astaxanthin did not reveal any clinical signs of toxicity, and did not affect survival, general condition or histological changes suggestive of any toxicity in liver, kidney and lung.

In another similar study, Tanaka et al. (1995b) also reported the suppression of azoxymethane-induced rat colon carcinogenesis in male F344 rats fed a diet containing astaxanthin for 34 weeks. In this study, one of the groups of rats (n=15) was exposed to the 500 mg astaxanthin/kg diet alone for 37 weeks. Rats were observed daily and weighed weekly until they reached 14 weeks of age and then every 4 weeks. Compared to the untreated control group, astaxanthin intake had no effect on mean body, liver and percentage liver weights. There were no clinical signs of toxicity, effects on survival, general health or histological changes suggestive of toxicity in liver, kidney or lung due to astaxanthin exposure.

### 2.5. Acute and Subchronic Studies with Biomass

In an acute oral study, *H. pluvialis* biomass (AstaREAL®, formerly marketed as AstaCarox®) containing 3% astaxanthin was administered to male and female Sprague-Dawley rats (Stewart et al., 2008). The $LD_{50}$ was found to be greater than 12 g/kg body weight. The results of this study suggest that the biomass (3% astaxanthin) is practically non-toxic. In another short-term toxicity study, daily gavage administration of the astaxanthin biomass (AstaREAL®) at a dose level of 6 g/kg body weight for 14 days did not result in any treatment-related deaths. Clinical observations and laboratory investigations did not reveal any treatment-related adverse effects. Similarly no treatment-related changes were noted at the post-mortem examination. As the astaxanthin

content of AstaREAL® used in this study was reported as 3%, it could be estimated that $LD_{50}$ of astaxanthin is greater than 360 mg/kg body weight (Stewart et al., 2008).

In a technical report on *H. pluvialis* and astaxanthin safety for human consumption, oral administration of *H. pluvialis* algae to rats at doses up to 5 g/kg body weight did not cause lethality (AstaFactor, 2004). In this report, three groups of rats (5/sex/group) were fed 5 g algal meal/kg body weight suspended in a 0.5% methylcellulose solution and animals were observed for signs of toxicity during a 13 day period. No signs of toxicity were noted. Similarly, post-mortem examination did not reveal any signs of toxicity. In yet another study mentioned in this report, *H. pluvialis* algae suspended in distilled water was orally administered to male and female mice at dosages ranging from 10 to 18 g/kg body weight. No mortalities occurred and no abnormalities were observed in the post-mortem examination.

In a subchronic study, Wistar rats (10/sex/group) were fed diets containing 0, 1, 5 and 20% of the biomass (AstaREAL®, formerly known as AstaCarox®) for 90 days (Stewart et al., 2008). This study was conducted according to the Organization for Economic Co-operation and Development (OECD) guidelines. Wistar strain rats (10/sex/group) were fed a standard rat diet containing 0, 10000, 50000 and 200000 ppm astaxanthin-rich biomass of *H. pluvialis* for 90 days. During the course of study, all animals were observed for morbidity and mortality, and clinical examinations were performed. Body weights and feed consumption were recorded at least weekly. Urine and blood samples for clinical evaluations (urinalysis, hematology, and plasma chemistry) were collected from all animals prior to scheduled necropsy. Over 40 tissues and organs were collected for histopathological examination. Compared to the control group, no treatment-related biologically significant effects of astaxanthin were noted on body weight or body weight gain. AstaREAL® feeding did not affect hematological parameters. In the high-dose group, slightly elevated alkaline phosphatase and changes in some urine parameters and an increase in kidney weight in both sexes were noted. Histopathology examinations did not reveal adverse effects except for a marginal increase in pigment in the straight proximal tubule of the kidney in 5/10 female rats treated with the high-dose. These changes were not considered to be toxicologically significant. Although the rats in the high-dose group received about 9% more fat, it is unlikely that this confounding factor significantly altered the outcome.

It should be noted that the dietary administration of the astaxanthin-rich biomass to rats was also associated with fur and feces staining in all treated groups (Stewart et al., 2008). Treatment was also associated with discoloration of the gastrointestinal tract in several intermediate and high-dose group animals of both sexes. The fur and feces coloration was likely due to the direct contact of the test article with the fur and gastrointestinal tract. In an earlier study (see section 2.4.), Takahashi et al. (2005) also reported orange color of stools, proventriculus mucous membrane, and caecal content following exposure to astaxanthin-rich *H. pluvialis* extract to rats. Histopathological observations of gastric mucosa did not reveal adverse findings. Hence these findings appear to be the result of adhesion of the test substance pigment. The NOAELs of the astaxanthin-rich biomass for male and female rats were determined as 14161 and 17076 mg/kg body weight/day, or 465 and 557 mg astaxanthin/kg body weight/day, respectively, the highest dose tested (Stewart et al., 2008).

In another study reported in Japanese, Ono et al. (1999) conducted a repeat-dose toxicity study of *haematococcus* color (prepared from dried algae using solvent extraction method) in male and female F344 rats. Four groups of rats (10/sex/group) were fed CRF-1 powder diet containing 0, 0.025, 0.075, and 0.25% *haematococcus* color (0, 0.5, 1.5, and 5% as the product, respectively) for 90 days. During the course of study no lethality was noted. There were no treatment-related changes in body weight gain or feed consumption. Serum biochemical analysis revealed a dose-related increase in cholesterol, but the differences were slight and not defined as an adverse effect. In the 90-day study by Onodera et al. (1997) with *Phaffia* color that contains higher levels of astaxanthin, no changes in cholesterol levels were noted. This suggests that the changes in cholesterol may not be related to astaxanthin. Besides cholesterol, no treatment-related effects were noted in hematological parameters, plasma chemistry, and organ weights. Similarly histopathological examinations did not reveal any abnormalities that could be ascribed to the treatment. The investigators concluded that ingestion of *haematococcus* color in the diet at the levels tested for 13 weeks did not cause any toxicological changes in F344 rats. The levels of astaxanthin in the *haematococcus* color were not mentioned.

### 2.6. Summary of Acute and Subchronic Studies

In acute toxicity studies of astaxanthin-rich extract (acetone extract) and synthetic astaxanthin, no rat deaths were noted at 2 g/kg body weight, the highest dose tested. Similarly, in acute toxicity studies of biomass, no lethality was noted at doses of 12 g/kg body weight. The results of these acute studies with biomass as well as with astaxanthin demonstrate that astaxanthin is practically non-toxic. The results from subchronic studies in rats following administration of astaxanthin (biomass, extract or synthetic) via gavage or in feed demonstrate that astaxanthin is without biologically significant adverse effects on a series of biochemical-related parameters. Similarly, in a dog study, the administration of astaxanthin did not reveal significant treatment-related adverse effects. Furthermore, the results from chemoprevention studies and in experiments where effects of astaxanthin on specific parameters were investigated support the safety of astaxanthin following long-term administration.

### 2.7. Teratogenicity/Reproductive Toxicity

In a repeat-dose toxicity and fertility study, Nishikawa et al. (1997) investigated the physiological and biochemical effects of β-carotene and astaxanthin in rats. For the toxicity study, six male Wistar rats were fed a diet containing 400 mg astaxanthin/kg (0.04%) for 41 days. No adverse effects of astaxanthin were observed on growth, clinical observations, organ weights and plasma levels of liver enzymes. For the fertility study, groups of Wistar rats (15/group/sex) were fed a diet containing astaxanthin (200 mg/kg; 0.02%) for 14 days. At the end of 14 days, animals were paired for mating. Feeding was continued for 42 days or until delivery. During the course of study, the rates of pregnancy and delivery, litter size, sex, and any teratogenic effects were determined. Astaxanthin administration did not affect fertility, litter or pup size. Further, astaxanthin did not cause any gross abnormalities in offspring.

For the color additive petition, Roche (1987) conducted embryotoxicity and teratology studies of a synthetic astaxanthin in rabbits. The teratogenicity study was conducted in accordance with the guidelines established by the US FDA and UK CSM

(Committee on the Safety of Medicines). In this study, time-mated rabbits were orally administered 0, 100, 200 and 400 mg astaxanthin/kg body weight/day from day 7 to 19 of gestation. All rabbits were euthanized on day 30 of gestation. Fetuses were removed by ovariohysterectomy, tested for viability (24 hours) and examined for macroscopic, skeletal, visceral and soft tissue abnormalities. Astaxanthin was well tolerated and no signs of overt toxicity were noted. No significant changes in body weight were noted between control and astaxanthin-treated animals. In the low (100 mg/kg body weight) and intermediate (200 mg/kg body weight) dose groups the measured reproductive and litter parameters, as well as course and outcome of pregnancy were unaffected. There was no evidence of astaxanthin-related malformations among the examined fetuses. In the high-dose (400 mg/kg body weight) treated animals, the only abnormal finding among the various measured parameters was a slight but non-significant increase in the incidence of resorptions (38%). The results of this study indicate that astaxanthin is unlikely to cause embryotoxic or teratogenic effects.

In a one generation study in rats, astaxanthin was tested for effects on fertility and general reproductive performance of the rat and on the *in utero* and postnatal development of the $F_1$-offspring to time of weaning (Roche, 1987). The study included the assessment of later development and of the reproductive capability of selected $F_1$-offspring retained beyond weaning. The study was conducted in accordance with the guidelines established by the US FDA and UK CSM. In this study astaxanthin (96% pure) was administered via oral gavage at doses 25, 100 or 400 mg/kg body weight/day to rats (12 male/group and 32 females/group) beginning 14 days prior to mating and continuing through gestation until euthanasia or weaning. Control rats (32/sex) received the vehicle (rape-oil, 2 mL/kg body weight) in a comparable regimen. About half of the mated females in each group were euthanized on "about" gestation day 14; while the remaining females were allowed to litter. $F_1$-pups of selected litters were evaluated for developmental indices during lactation. On lactation day 23, selected weanlings were retained for learning and memory testing or the assessment of their reproductive capability. The results of the study were summarized as follows:

*P-generation*: No substance-related mortality in males or females was observed in any of the dosage groups. The body weight gain of both P-males and P-females in all experimental groups matched that of the concurrent controls. The percentage of males which mated their partners, as well as the ratio of mated to pregnant females and the median precoital time were comparable among all groups. Up to 400 mg/kg body weight, the highest dose tested, the reproductive parameters of females euthanized between gestation days 14 and 16 were within normal limits.

*$F_1$-generation*: In all experimental groups, the litter parameters such as the body weight gain of pups, the time of onset of developmental landmarks and the learning and memory ability matched that of the controls. The neonatal mortality of the $F_1$-generation in the highest dosage group (400 mg/kg body weight) was at the upper limit of the biological range (25.6%). However, there was no statistical significance for this finding and no dose-relationship was evident. Therefore a substance-related impairment of pup viability was considered (by the petitioner) to be very unlikely. In all dosage groups, the macroscopic and soft tissue examination of pups found dead during lactation showed isolated findings which were not considered (by the petitioner) to be treatment-related.

These included hematoma in the lung, empty stomach, and dilated renal pelvis and ureter. The gross examination of weanlings for malformations, as well as for liver and kidney weights, yielded normal findings. One neonate in the low-dose group (25 mg/kg body weight) exhibited unilateral anophtalmia. This isolated anomaly was not considered to be substance-related. The reproductive capabilities of $F_1$ animals was not adversely affected in any of the experimental groups. The number of $F_2$-pups which died or were cannibalized between lactation days 1 and 4 was unusually high in all groups, including controls, and therefore was considered to have resulted from other than treatment-related influences.

Based on the results of the multi-generation studies, the petitioner concluded that the no-effect-level of astaxanthin given by oral gavage to male and female rats during gametogenesis, mating, gestation and lactation was 400 mg/kg body weight/day, the highest dose tested in the study (Roche, 1987).

### 2.8. Genotoxicity

#### 2.8.1. Studies with Extract or Synthetic Astaxanthin

Takahashi et al. (2005) investigated the potential mutagenic effects of an astaxanthin-rich extract at concentrations up to 5000 μg/plate in the Ames test using *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537 in the presence or absence of metabolic activation. The results did not reveal any biologically significant increase in the number of revertant colonies in any tester strains following the treatment with the test article and it was concluded that the astaxanthin-rich extract was not mutagenic.

Roche (1987) examined the potential mutagenicity of synthetic astaxanthin. Although experimental details were not available, the studies were summarized in a FDA document. In the Ames test, astaxanthin at concentrations ranging from 30 to 5000 μg/plate did not induce mutation in *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537 and TA1538 in the presence or absence of rat liver homogenate fraction. In the micronucleus test, astaxanthin in the form of 10% gelatin beadlets was administered orally to mice at 30 hours and again at 6 hours prior to euthanasia. The dosage of astaxanthin was 500, 1000, and 2000 mg/kg body weight of the 10% gelatin beadlets. Astaxanthin administration did not result in an increase in micronuclei and it was concluded that astaxanthin at the doses tested induces neither chromosome breaks nor mitotic disjunction *in vivo.*

#### 2.8.2. Studies with Biomass

The mutagenic effects of the powder form of homogenized and dried algal cells of *H. pluvialis* were tested in the Ames test using *S. typhimurium* strains TA100, TA1535, TA98, TA1537, TA1538, and *Escherichia coli* WP2 uvr A pKm101 (Scantox, 1998a). The study was conducted in accordance with OECD guidelines. Two independent tests were performed using five different concentrations of the powder in the range of 313-5000 μg/plate in the presence and absence of an S-9 metabolic activation. The results demonstrated that *H. pluvialis* was non-mutagenic in the Ames test.

In an *in vitro* mammalian cell gene mutation test, the powder form of homogenized and dried algal cells of *H. pluvialis* was tested using mouse lymphoma

(L5178Y) cells in the presence and absence of S-9 mix (Scantox, 1998b). The study was conducted in accordance with OECD guidelines. The test was performed in two independent experiments using a range of concentrations up to 5000 μg/ml of test material. In the absence of S-9 mix, the cells were treated for 4 hours in the first test and for 24 hours in the second test. In the presence of S-9 mix, the cells were treated for 3 hours in both tests. Compared to negative control cultures, no biologically or statistically significant increases in mutation frequency was noted in cultures treated with the test material. The results of these experiments demonstrated that *H. pluvialis* was not mutagenic.

In an *in vivo* mouse micronucleus test, the genotoxic potentials of the powder form of homogenized and dried algal cells of *H. pluvialis* were investigated (Scantox, 1998c). The study was conducted in accordance with OECD guidelines. Groups of mice (5/sex/group) were administered once, via gavage, the *H. pluvialis* powder in distilled water (vehicle) at a dose level of 2000 mg/kg body weight. The control group received the vehicle alone while the positive control group received cyclophosphamide (20 mg/kg body weight). Bone marrow samples were collected at 24 and 48 hours after dosing. Administration of *H. pluvialis* powder did not cause any statistically significant reduction in the frequency of polychromatic erythrocytes among total erythrocytes suggesting that there were no adverse effects on the bone marrow. Similarly, no biologically or statistically significant increase in the frequency of micronucleated polychromatic erythrocytes was seen. In the positive control group, a significant increase in the frequency of micronucleated polychromatic erythrocytes was noted. The results of this study demonstrated that *H. pluvialis* powder did not have mutagenic/clastogenic activity in mouse.

In a technical report, *H. pluvialis* algae meal was reported as without any mutagenic effect when tested in standard tests systems using *S. typhimurium* strains TA100, TA1535, TA98, TA1537, TA1538, and *E. coli* WP2 uvr A (AstraFactor, 2008). In these experiments, the meal was formulated in a 50 mg/mL solution of dimethyl sulfoxide. The formulation was spread onto petri dishes in the presence of the microbial cultures. The algal meal did not reveal any increase in revertants. The positive controls (mutagenic agents) showed a predictable increase in the number of reverent colonies in every case, compared to the solvent control.

### 2.9. Anti-oxidant/pro-oxidant Activity

Goto et al. (2001) investigated the effects of the carotenoids β-carotene and astaxanthin on the peroxidation of liposomes induced by ADP and $Fe^{2+}$. Astaxanthin was about 2-fold more effective than β-carotene in inhibiting the production of lipid peroxides. The difference in the modes of destruction of the conjugated polyene chain between β-carotene and astaxanthin suggested that the conjugated polyene moiety and terminal ring moieties of the more potent astaxanthin trapped radicals in the membrane and both at the membrane surface and in the membrane, respectively, whereas only the conjugated polyene chain of β-carotene was responsible for radical trapping near the membrane surface and in the interior of the membrane. These investigators suggested that the efficient antioxidant activity of astaxanthin is due to the unique structure of the terminal ring moiety.

In another study, Martin et al. (1999) investigated the anti-oxidant and pro-oxidant activities of carotenoids. The results of these investigations lead to the conclusion that the different chemical anti- and pro-oxidant behavior of the carotenoids is caused by the different structure of their end groups, their chain length (minor importance) and the number and position of methyl groups. β-Carotene is able to react as a hydrocarbon with active allylic hydrogen atoms that can be removed by radicals. On the other hand, β-carotene also binds to peroxyl radicals. Both processes combine to produce reactions with the concomitant formation of epoxides and carbonyl compounds. It is possible to develop a sequence of radical abstraction and oxygen addition reactions as well as cleavage reactions resulting in a radical chain reaction. In contrast, astaxanthin does not enter these pathways and is unlikely to act as a pro-oxidant. In another study, Nielsen et al. (1998) reported that astaxanthin may be a better antioxidant against light-induced, singlet oxygen mediated oxidations than β-carotene and zeaxanthin.

The above described studies suggest that astaxanthin would not be a pro-oxidant. Intervention studies conducted with β-carotene have reported that supplemental β-carotene may enhance lung tumorigenesis in cigarette smokers (ATBC, 1994; Omenn et al., 1996a, 1996b), potentially via altered retinoid signaling (Paolini et al., 1999; Wang et al., 1999). Unlike β-carotene, astaxanthin is a non-provitamin A carotenoid, and is not expected to directly alter retinoid signaling. Additionally, as mentioned earlier, several experimental studies with astaxanthin have reported chemopreventive effects. Furthermore, toxicology studies conducted with astaxanthin have reported no adverse toxicological effects, and no mutagenic activity of astaxanthin has been reported *in vitro* or *in vivo*.

## 3. SUMMARY

Astaxanthin, a carotenoid, is found in the red pigment of crustacean shells (i.e. crabs, shrimp), salmon, and the asteroidean. Unlike some carotenoids, astaxanthin is not converted to Vitamin A in humans. As astaxanthin is naturally present in commonly consumed seafood such as salmon, red fish, shrimp, krill and lobster, astaxanthin has a long history of being a component of the human diet. Because of its perceived health benefits, astaxanthin is commonly marketed as a dietary supplement without evidence of adverse effects. Use of astaxanthin and *Haematococcus* algae meal is approved by FDA as a color additive to salmonid fish feed at levels up to 80 mg/kg feed to enhance the color of the flesh. It has been claimed that carry-over of astaxanthin from farmed salmon into the human food chain is beneficial to human health (Baker and Gunther, 2004). Based on a European Union report, for consumers with high intakes of fish and seafood, the estimated daily intake of astaxanthin ranged from 1.6 to 4.1 mg/day. In the U.S., in the extreme case it was considered that all fish consumed is Sockeye salmon (upper bound scenario), the 90$^{th}$ percentile daily intake of astaxanthin is estimated as 6.87 mg/person.

Fuji Chemical Industry Co. has developed a process to manufacture standardized astaxanthin esters from *H. pluvialis.* Fuji intends to use an astaxanthin-rich extract containing astaxanthin esters as a nutrient in Baked Goods, Beverages, Cereals, Chewing Gum, Coffee and Tea, Dairy Product Analogs, Frozen Dairy Desserts and Mixes, Hard

Candy, Milk Products, Processed Fruits and Fruit Juices, Processed Vegetables and Vegetable Juices, and Soft Candy at levels of 0.5 mg astaxanthin *per* serving (reference amounts customarily consumed, 21CFR 101.12). Based on the intended use levels and using CSFII 2003-04 (USDA, 2004) database, the use of astaxanthin esters in the above mentioned food categories resulted in an estimated daily intake of astaxanthin for "users only" at mean and 90$^{th}$ percentile of 3.2 mg/person and 5.4 mg/person (0.1 mg/kg body weight/day), respectively.

As a fat-soluble compound, astaxanthin digestion and intestinal absorption is closely associated with fatty acid uptake, transport and delivery. Following oral administration, astaxanthin is absorbed but its bioavailability is low. Its esterified form is relatively more bioavailable compared to the free form. Esterified astaxanthin was not detected in plasma. The absorption of astaxanthin is enhanced by incorporation into a lipid formulation. Available evidence indicates that astaxanthin is not metabolized by the microsomal xenobiotic-metabolizing enzymes. Length of exposure to astaxanthin is not related to the plasma concentration and astaxanthin is unlikely to be accumulated in the body. Orally administered astaxanthin is incorporated into chylomicrons, and distributed between VLDL, HDL and LDL fractions in the circulation. Limited *in vivo* experiments revealed the presence of four astaxanthin metabolites. Metabolism-related studies suggest that astaxanthin is unlikely to affect cytochrome P450 enzymes in humans.

The results of acute oral toxicity ($LD_{50}$ > 5 to 12 g/kg body weight) studies of *H. pluvialis* biomass suggest that the biomass which is known to contain astaxanthin (~1-3%) is practically non-toxic. In a subchronic toxicity study of *H. pluvialis* biomass (3% astaxanthin), the NOAELs of the biomass for male and female rats were found to be 14161 and 17076 mg/kg body weight/day, or 465 and 557 mg astaxanthin/kg body weight/day, respectively, the highest dose tested. As the intended uses of astaxanthin esters will result in an estimated intake of 0.1 mg/person/day, the results of subchronic study provide a 5000-fold safety margin. No mortality was noted in animals following administration of either an astaxanthin-rich extract or synthetic astaxanthin at doses of 2 g/kg body weight. In a subchronic toxicity study, daily oral (gavage) administration of up to 50 mg astaxanthin/kg body weight/day (AstaREAL®; acetone extract) to Sprague-Dawley rats for 90 days did not result in any treatment-related adverse effects. The NOAEL was 50 mg astaxanthin/kg body weight/day, the highest dose tested. Administration of synthetic astaxanthin in gelatin beadlets to rats at a dose of 1250 mg/kg body weight/day or to dogs at a dose up to 160 mg/kg body weight/day for 13 weeks did not reveal any adverse effects. Thus, compared to the estimated daily intake resulting from the intended uses of astaxanthin there is over a 12500-fold safety factor from the NOEL in rats and a 1600-fold safety factor from the NOEL in dogs.

In *in vitro* and *in vivo* mutagenicity studies as evaluated by the Ames test, a mammalian gene mutation test, and a mouse micronucleus test, astaxanthin in the form of biomass, in the extract or synthetic form did not reveal any genotoxic effects. In a fertility testing study, exposure of male and female rats to astaxanthin for 14 days before mating and for 24 days after pregnancy did not affect fertility, litter or pup size; there were no gross abnormalities in offspring. In an embryotoxicity and teratology study, administration of synthetic astaxanthin (0, 100, 200 and 400 mg astaxanthin/kg body weight/day) to rabbits from gestation day 7 to 19 did not reveal any significant adverse

effects. In a one generation study, the no-effect-level of astaxanthin given by oral gavage to male and female rats during gametogenesis, mating, gestation and lactation was determined as 400 mg/kg body weight/day, the highest dose tested. Multiple clinical studies (>25) with over 500 subjects revealed that oral administration of astaxanthin at doses ranging from 2 to 40 mg/day for a period of 2 weeks to 12 months is without any adverse effect. In the majority of these clinical studies, astaxanthin was derived from *H. pluvialis*.

There is sufficient qualitative and quantitative scientific evidence, including both human and animal data, to determine safety-in-use of astaxanthin-rich extract derived from *H. pluvialis* algae. The safety data on astaxanthin includes repeat-dose (3 month) studies in rats and dogs, teratogenicity/reproductive toxicity studies in rats and rabbits, *in vitro* and *in vivo* genotoxicity studies, and over 25 human clinical trials. The safety determination of astaxanthin is based on the totality of available evidence such as human clinical observations and a variety of animal experimental data which further corroborate the safety in use. In human clinical trials, no adverse effects of astaxanthin were noted at doses of up to 40 mg/day. Results from a subchronic rat study show that astaxanthin (AstaREAL®) had no adverse effects at dose levels up to 50 mg astaxanthin/kg body weight/day, the highest dose tested. In another subchronic study, no adverse effects of astaxanthin were noted at doses up to 465 and 557 mg astaxanthin/kg body weight/day in male and female rats, respectively. The results from these animal studies provide a margin of safety ranging from 500 to 5000 fold above the estimated daily intake of astaxanthin resulting from the intended uses in specified foods. Based on the available information discussed above, the intake of astaxanthin esters in the diet from certain specified foods identified above at an estimated daily 90$^{th}$ percentile intake for "users only" of 5.4 mg astaxanthin/person (0.1 mg/kg body weight/day) is not considered to pose any safety concerns.

The safety of astaxanthin is supported by:

- Astaxanthin is naturally found in regularly consumed foods such as salmon, red fish, shrimp, krill and lobster.
- The long-term repeat dose exposure of astaxanthin did not result in its bioaccumulation.
- Animal and *in vitro* studies support the safety of astaxanthin.
- In multiple human clinical studies (>25), the safety of astaxanthin was confirmed at doses of up to 40 mg/day. Results from these trials provide an ample margin of safety above the resulting intake at the 90$^{th}$ percentile from the intended uses of astaxanthin.
- There is no evidence that consumption of astaxanthin in foods or as a dietary supplement has any cumulative effect that would affect its safety.

Astaxanthin has been marketed as a dietary supplement for approximately 10 years without any adverse effects. The typical recommended doses of astaxanthin as a dietary supplement are 6 mg/day (range of 2 to 12 mg/day). The intended use of astaxanthin esters will result in a daily estimated 90$^{th}$ percentile intake for "users only" of 5.4 mg astaxanthin/person (0.1 mg/kg body weight/day). In addition to the exposure from the intended uses as a nutrient in the specified foods, astaxanthin is also regularly

consumed in the diet due to its natural presence in certain foods. In an upper bound intake estimate scenario, the 90th percentile daily intake of astaxanthin from its natural presence in food is estimated as 6.87 mg/person. The 90th percentile intake of astaxanthin from its intended uses is approximately 7-fold lower than the safe levels (40 mg/day) noted in human clinical trials. The combined intake of astaxanthin from its intended uses and from its natural presence in food is below the safe levels identified based on human clinical trails and animal studies.

On the basis of scientific procedures[2] including knowledge from a history of exposure from natural sources, the consumption of astaxanthin esters as an added food ingredient is considered safe at levels up to 5.6 mg/day. The intended uses are compatible with current regulations, *i.e.,* astaxanthin esters can be used at the levels specified above in Baked Goods, Beverages, Cereals, Chewing Gum, Coffee and Tea, Dairy Product Analogs, Frozen Dairy Desserts and Mixes, Hard Candy, Milk Products, Processed Fruits and Fruit Juices, Processed Vegetables and Vegetable Juices, and Soft Candy produced according to current Good Manufacturing Practices (cGMP) where not otherwise precluded by a standard of identity.

[2] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

## 4. CONCLUSION

Based on a critical evaluation of the publicly available data summarized herein, the Expert Panel members whose signatures appear below, have individually and collectively concluded that astaxanthin esters derived from *Haematococcus pluvialis*, meeting the specifications cited herein, and when used as a nutrient in Baked Goods, Beverages, Cereals, Chewing Gum, Coffee and Tea, Dairy Product Analogs, Frozen Dairy Desserts and Mixes, Hard Candy, Milk Products, Processed Fruits and Fruit Juices, Processed Vegetables and Vegetable Juices, and Soft Candy at use levels of 0.5 mg astaxanthin *per* serving (reference amounts customarily consumed, 21 CFR 101.12) and when not otherwise precluded by Standards of Identity are safe.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that astaxanthin esters, when used as described, are GRAS based on scientific procedures.

**Signatures**

| | |
|---|---|
| Stanley M. Tarka, Jr. Ph.D. | Date |
| John A. Thomas, Ph.D., F.A.T.S., D.A.T.S. | Date |
| Madhusudan G. Soni, Ph.D., F.A.C.N. | Date |

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## 6. APPENDIX I

Analytical data from different manufacturing lots (Information provided by Fuji)

**Compositional Analysis of Crude Oil Extracted with Supercritical $CO_2$**

| | L (b)(4) | Lot No. | Lo (b)(4) | |
|---|---|---|---|---|
| | **Composition (%, w/w)** | **Composition (%, w/w)** | **Composition (%, w/w)** | **Analytical method** |
| Acylglycerol | | | | JFRL method |
| Triacylglycerol | 54.7 | 54.8 | 50.9 | |
| Diacylglycerol | 17.3 | 17.2 | 18.5 | |
| Monoacylglycerol | N.D. | N.D. | 1.0 | |
| Subtotal | 72.0 | 72.0 | 70.4 | |
| Free Fatty Acid | 0.70 | 0.39 | 0.55 | JOCS 3.3.5; J.P. 2.02 |
| Carotenoids | | | | Reverse phase HPLC |
| Astaxanthin | 15.5 | 15.5 | 16.2 | |
| Others | 1.3 | 1.0 | 1.1 | |
| Subtotal | 16.8 | 16.5 | 17.3 | |
| Tocopherols | 2.4 | 2.4 | 2.9 | Normal phase HPLC |
| Phospholipids | 0.048 | 0.033 | 0.047 | JOCS 2.4.11 |
| Moisture | N.D. | 0.20 | 0.09 | JP 2.48 |
| **Total** | **91.9** | **91.3** | **91.1** | |

JFRL = Japan Food Research Lab; JP = Japanese Pharmacopoeia; JOCS = Japan Oil Chemists' Society; ND = Not detected

## 6.1. APPENDIX II

Analytical data from different manufacturing lots (Information provided by Fuji)

**Composition Analysis of Crude Oil Extracted with Acetone**

| | Lot No. (b)(4) | Lot No. (b)(4) | Lot No. (b)(4) | |
|---|---|---|---|---|
| | Composition (%, w/w) | Composition (%, w/w) | Composition (%, w/w) | Analytical method |
| Acylglycerol | | | | JFRL method |
| Triacylglycerol | 51.5 | 49.4 | 51.8 | |
| Diacylglycerol | 16.9 | 16.0 | 16.4 | |
| Monoacylglycerol | ND | ND | ND | |
| Subtotal | 68.4 | 65.4 | 68.2 | |
| Free Fatty Acid | 0.25 | 0.41 | 0.32 | JOCS 3.3.5; J.P. 2.02 |
| Carotenoids | | | | Reverse phase HPLC |
| Astaxanthin | 15.7 | 16.3 | 14.8 | |
| Others | 0.5 | 0.6 | 0.8 | |
| Subtotal | 16.2 | 16.9 | 15.6 | |
| Tocopherols | 6.2 | 5.8 | 6.3 | Normal phase HPLC |
| Phospholipids | 0.17 | 0.17 | 0.26 | JOCS 2.4.11 |
| Moisture | 0.29 | 0.39 | 0.02 | JP 2.48 |
| **Total** | **91.2** | **88.7** | **90.6** | |

JP= the Japanese Pharmacopoeia; JOCS= Japan Oil Chemists' Society

## 6.2. APPENDIX III

Analytical data from five manufacturing lots (Information provided by Fuji)

**Analysis of Crude Oil Extracted with Supercritical $CO_2$**

| | Standard/typical* | (b)(4) | (b)(4) | (b)(4) | (b)(4) | (b)(4) |
|---|---|---|---|---|---|---|
| Appearance | Dark red viscous oil | Complies | Complies | Complies | Complies | Complies |
| Astaxanthin | NLT 10% (As free form by HPLC) | 10.9% | 10.8% | 11.8% | 11.0% | 11.0% |
| Pheophorbide | Less than 20 mg/dL | Complies | Complies | Complies | Complies | Complies |
| Heavy metals | Less than 10 ppm | Complies | Complies | Complies | Complies | Complies |
| ArsenicLead | Less than 2 ppm | Complies | Complies | Complies | Complies | Complies |
| Lead | Less than 1 ppm | Complies | Complies | Complies | Complies | Complies |
| Mercury | Less than 1 ppm | Complies | Complies | Complies | Complies | Complies |
| Cadmium | Less than 1 ppm | Complies | Complies | Complies | Complies | Complies |
| Microbiological assays | | | | | | |
| Total plate count | Less than 1000 cfu/g | Complies | Complies | Complies | Complies | Complies |
| Yeast and Mold | Less than 100 cfu/g | Complies | Complies | Complies | Complies | Complies |
| Coliform bacteria | Negative/g | Negative | Negative | Negative | Negative | Negative |
| *Staphylococcus aureus* | Negative/g | Negative | Negative | Negative | Negative | Negative |
| *Salmonella* | Negative/g | Negative | Negative | Negative | Negative | Negative |

*Standard specifications for marketed product

**Analytical methods used in the above determinations:**

| | |
|---|---|
| Appearance | Visual test |
| Astaxanthin | UV-VIS (JP[1] 2.24, E=2100 (in acetone)) & HPLC (JP[1] 2.01, Reversed-phase column, Mobile phase; methanol/t-butyl-methyl-ether/1% phosphoric acid gradient solution) See appendix V |
| Pheophorbide | Kanshoku No.99[2] |
| Heavy metals | ICP-MS (sum of Pb, As, Cd and Hg) - modified NMKL[3] 161 |
| Arsenic | ICP-MS - modified NMKL[3] 161 |
| Lead | ICP-MS - modified NMKL[3] 161 |
| Mercury | AFS- modified NMKL[3] 170 |
| Cadmium | ICP-MS - modified NMKL[3] 161 |
| Total plate count | NMKL[3] 86,4 2006 |
| Yeast and Mold | NMKL[3] 98-modified |
| Coliform bacteria | NMKL[3] 44, 6, 2004 modified according to Ph. Eur. method 2.6.13 |
| *Staphylococcus aureus* | NMKL[3] 66 modified according to Ph. Eur. method 2.6.13 |
| *Salmonella* | NMKL[3] 125, 4 2005 |

Extraction Method; Super critical fluid extraction

Pesticides and organic solvents are not used under the manufacturing process.

[1]Japanese Pharmacopoeia

[2]Notice from Environmental Health Bureau-Ministry of Health and Welfare dated May 8th, 1981

[3]Nordic Committee on Food Analysis

## 6.3. APPENDIX IV

Analytical results from five manufacturing lots (Information provided by Fuji)

**Analysis of Crude Oil Extracted with Acetone**

| | Standard/typical* | (b)(4) | (b)(4) | (b)(4) | (b)(4) | (b)(4) |
|---|---|---|---|---|---|---|
| Appearance | Dark red viscous oil | Complies | Complies | Complies | Complies | Complies |
| Astaxanthin | NLT 10% (As free form by HPLC) | 11.4% | 11.0% | 11.1% | 10.0% | 11.0% |
| Pheophorbide | Less than 20 mg/dL | Complies | Complies | Complies | Complies | Complies |
| Heavy metals | Less than 10 ppm | Complies | Complies | Complies | Complies | Complies |
| Arsenic | Less than 2 ppm | Complies | Complies | Complies | Complies | Complies |
| Lead | Less than 1 ppm | Complies | Complies | Complies | Complies | Complies |
| Mercury | Less than 1 ppm | Complies | Complies | Complies | Complies | Complies |
| Cadmium | Less than 1 ppm | Complies | Complies | Complies | Complies | Complies |
| Microbiological assays | | | | | | |
| Total plate count | Less than 1000 cfu/g | Complies | Complies | Complies | Complies | Complies |
| Yeast and Mold | Less than 100 cfu/g | Complies | Complies | Complies | Complies | Complies |
| Coliform bacteria | Negative/g | Negative | Negative | Negative | Negative | Negative |
| *Staphylococcus aureus* | Negative/g | Negative | Negative | Negative | Negative | Negative |
| *Salmonella* | Negative/g | Negative | Negative | Negative | Negative | Negative |
| Residual acetone | Less than 5 ppm | Complies | Complies | Complies | Complies | Complies |

*Standard specifications for marketed product

**Analytical methods used in the above determinations:**

| | |
|---|---|
| Appearance | Visual test |
| Astaxanthin | UV-VIS (JP[1] 2.24, E=2100 (in acetone)) & HPLC (JP[1] 2.01, Reversed-phase column, Mobile phase; methanol/t-butyl-methyl-ether/1% phosphoric acid gradient solution) |
| Pheophorbide | Kanshoku No.99[2] |
| Heavy metals | As Pb, JP[1] 1.07, sodium sulfide-colorimetry |
| Arsenic | JP[1] 1.11, DDTC-Ag-colorimetry |
| Lead | AHS[3] 1.1.6, Atomic Absorption Spectrometry |
| Mercury | AHS[3] 2.4.1, CV-Atomic Absorption Spectrometry |
| Cadmium | AHS[3] 1.1.6, Atomic Absorption Spectrometry |
| Total plate count | JP[1] 4.05 |
| Yeast and Mold | JP[1] 4.05 |
| Coliform bacteria | AHS[3] 1.2.1, BGLB method |
| *Staphylococcus aureus* | JP[1] 4.05 |
| *Salmonella* | JP[1] 4.05 |
| Residual acetone | GC (JP[1] 2.02) |

Extraction Method; Acetone extraction

Pesticides are not used under the manufacturing process.

[1]Japanese Pharmacopoeia

[2]Notice from Environmental Health Bureau-Ministry of Health and Welfare dated May 8th, 1981

[3]Official Methods of Analysis in Health Science

## 6.4. APPENDIX V

# Spectrophotometric and HPLC Analysis Method for Determining Astaxanthin Content in AstaREAL® L10

**(Attached separately)**

## 6.5. APPENDIX VI

# ESTIMATED DAILY INTAKE OF ASTAXANTHIN BY THE U.S. POPULATION FROM PROPOSED FOOD-USES

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# Appendix V
## Spectrophotometric and HPLC Analysis Method for Determining Astaxanthin Content in AstaREAL® L10

1.0 *Reagents and Equipment*

*0.05M Tris-HCl buffer (pH7.0)
*Cholesterol esterase: Wako Pure Chem., cat# 037-11221
*Trans-beta-apo-8'-carotenal, SIGMA cat# A9956; internal standard for HPLC analysis
*Astaxanthin, SIGMA cat# A9335, analytical standard
*1% (v/v) phosphoric acid solution
*Acetone, Spectrophotometric grade
*Hexane, HPLC grade
*Petroleum ether
*Methanol, analytical grade
*MTBE: t-butyl-methyl-ether, spectrophotometric grade
*Sodium sulphate decahydrate
*Sodium sulphate anhydrous
*10mL centrifuge tubes
*30mL volumetric flasks
*100mL volumetric flasks
*200mL volumetric flasks
*0.45um syringe filter
*Water bath
*Analytical balance
*Centrifuge
*Sonicator
*Spectrophotometer
*HPLC equipped with a UV/VIS detector
*HPLC column: YMC-Carotenoid™ S5 micron, 250mm length x 4.6mm dia.

2.0 *Cholesterol Esterase Solution for hydrolysis of Astaxanthin Esters*

a) Dissolve an accurately weighed quantity of cholesterol esterase (Wako Pure Chem., Cat #: 037-11221) in 50 mM Tris-HCl (ph 7.0) having a known concentration of 4units per mL. prepare this solution fresh daily.

3.0 *Internal Standard Preparation*

a) Accurately weigh about 7.5 mg of *trans-beta-apo*-8'-Carotenal (SIGMA, Cat #: A9956, >20 %(UV-VIS) Apocarotenal. Hereinafter referred to as I.S.) and transfer into a 200-mL volumetric flask.
b) Dissolve in acetone, dilute with acetone to volume, and mix.

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4.0 *Standard Preparation*

a) Transfer about 5 mg each of Astaxanthin reagents (SIGMA, Cat #: A9335) to a 200-mL volumetric flask, dissolve in about 100 mL of acetone sonicating for a minute, and allow to equilibrate to ambient temperature for 15 minutes.
b) Dilute with acetone to volume and mix (*Standard stock solution*).
c) Pipette 3.0 mL of *Standard stock solution* to a 30-mLvolumetric flask, dilute with acetone to volume, and mix (*Standard solution A*).
d) Pipette 3.0 mL of *Standard stock solution* and 10.0 mL of *Internal standard solution* to a 30-mL volumetric flask, dilute with acetone to volume, and mix (*Standard solution B*).

5.0 *Assay Preparation*

a) Transfer an accurately weighed about 30 mg of AstaREAL® L10 to a 100-mL volumetric flask, dilute with acetone to volume, and mix.
b) Pipette 2.0 mL to a 20-mL volumetric flask, dilute with acetone to volume, and mix (*Assay solution A*).
c) Pipette 3.0 mL of *Assay solution A* to a 10-mL glass centrifuge tube, add 1.0 mL of *Internal standard solution*, and mix.
d) Preheat in water bath or on block heater at 37 °C for 2 minutes, add 3.0 mL of *Cholesterol esterase solution*, and mix gently with inverting.
e) Allow to react at 37 °C for 45 minutes. Mix gently with inverting every 10 minutes while reaction.
f) Add 1 g of sodium sulfate decahydrate and 2 mL of petroleum ether, vortex for 30 seconds, and centrifuge at 3,000 rpm for 3 minutes.
g) Transfer the petroleum ether layer to a 10-mL glass centrifuge tube containing 1 g of sodium sulfate anhydrate.
h) Evaporate the petroleum ether layer in the stream of inert gas at room temperature, add 3 mL of acetone, sonicate to dissolve, and filtrate (*Assay solution B*).

6.0 *Procedure_Spectrophotometric Analysis Method for Total Carotenoids Content*

a) Determine the absorbance of *Assay solution A* at 474nm against an acetone blank on the spectrophotometer.
b) Calculate the Total Carotenoids Content (%) in AstaREAL® L10 taken by the formula:

$$A_{Aa} * 1000 / 210 / W * 100$$

in which $A_{Aa}$ is the absorbance of *Assay solution A*, 210 is absorbance of a 1 (mg/mL) carotenoids solution in acetone, in a 1 cm cuvette at 474 nm, $W$ is the weight, in mg, of the specimen taken for *Assay preparation*.

c) Refer to Appendix A. Data I: Figure 1., for typical absorbance profile for AstaReal® L10 and astaxanthin standard.

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7.0 *Procedure_Reversed-phase HPLC Analysis Method for Astaxanthin Content*

a) Determine the absorbance of *Standard solution A* at 474 nm, using acetone as the blank.
b) Analyze an aliquot of *Standard solution B* and *Assay solution B* by HPLC under the following conditions:

Table-1. *HPLC conditions*:

Detector: UV/VIS detector, at 474 nm,
Column: YMC-Carotenoid™ S5μ, 4.6 x 250 mm,
Column temp.: 25 °C,
Flow rate: 1.0 mL / minute,
Injection vol.: 20 μL,
Mobile phase: Methanol, *t*-Butylmethylether, 1 % Phosphoric acid aqueous,
Mobile phase formula (%) is as follows:

| Time (min.) | Methanol | *t*- Butylmethylether | 1 % Phosphoric acid aqueous |
|---|---|---|---|
| 0 | 81 | 15 | 4 |
| 15 | 66 | 30 | 4 |
| 23 | 16 | 80 | 4 |
| 27 | 16 | 80 | 4 |
| 27.1 | 81 | 15 | 4 |
| 35 | 81 | 15 | 4 |

Table-2. *Retention time for Identification*:

| Components | Retention time (min.) |
|---|---|
| 13-*cis*-astaxanthin: | 9 |
| *trans*-astaxanthin: | 10 |
| 9-*cis*-astaxanthin: | 14 |
| *trans-beta-apo*-8'-carotenal: (*Internal standard*) | 16 |

c) Refer to Appendix A. Data II: Figure 2., and Figure 3., for typical chromatograms for AstaREAL® L10 with internal standard.
d) Calculate the concentration, in mg per mL, of astaxanthin in the *Standard solution A* taken by the formula:

$$A_{Sa} / 210$$

in which $A_{Sa}$ is the absorbance of *Standard solution A*, and 210 is absorbance of a 1 (mg/mL) astaxanthin solution in acetone, in a 1 cm cuvette at 474 nm.

e) Calculate the ratios of peak responses of total astaxanthin to I.S. obtained from the *Assay solution B* and *Standard solution B* taken by the formula:

$$(1.3P_{13\text{-}cis} + P_{trans} + 1.1P_{9\text{-}cis}) / P_{I.S.}$$

in which $P_{13\text{-}cis}$, $P_{trans}$, $P_{9\text{-}cis}$ and $P_{I.S.}$ are the peak responses of 13-*cis*-, *trans*-, 9-*cis*- astaxanthin isomers and I.S., respectively, and 1.3 and 1.1 are the relative response coefficients of 13-*cis*-, and 9-*cis*-astaxanthin to *trans*-astaxanthin, respectively.

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f) Calculate the Astaxanthin Content (% w/w) in AstaREAL® L10 taken by the formula:

$$C_{Sa}\,(R_{Ab} / R_{Sb}) * 1000 / W * 100$$

in which $C_{Sa}$ is the concentration, in mg per mL, of astaxanthin in the *Standard solution A*, $W$ is the weight, in mg, of the specimen taken for *Assay preparation*, and $R_{Ab}$ and $R_{Sb}$ are the ratios of the peak responses of total astaxanthin to I.S. obtained from the *Assay solution B* and the *Standard solution B*, respectively.

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**Appendix A. Data I**

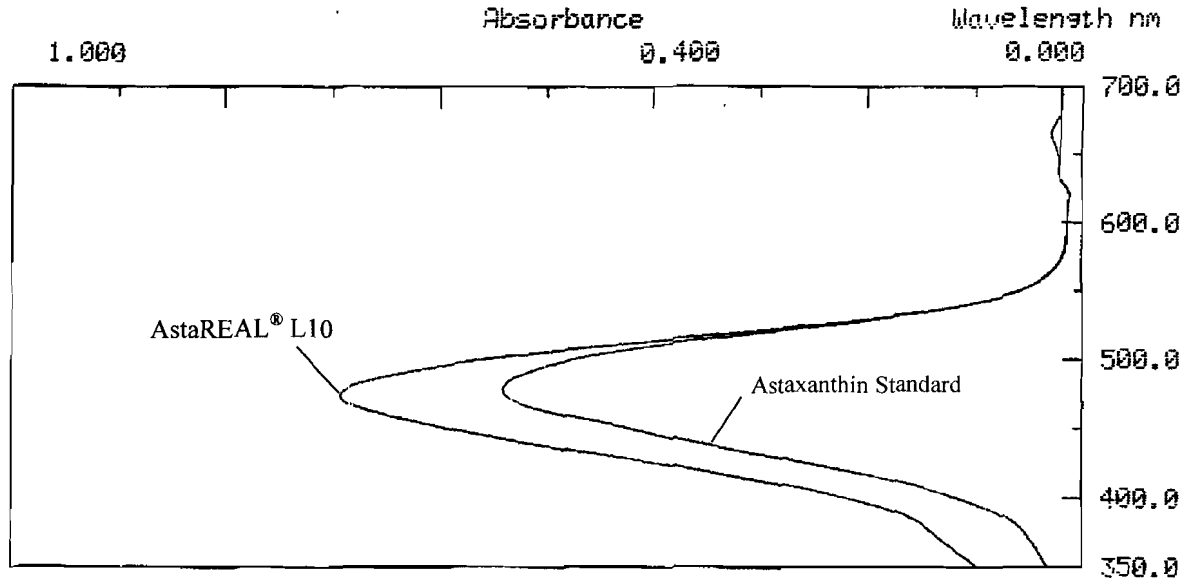


Fig-1. Spectrophotometric profiles of AstaREAL® L10 and astaxathin standard.



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## Appendix A. Data II

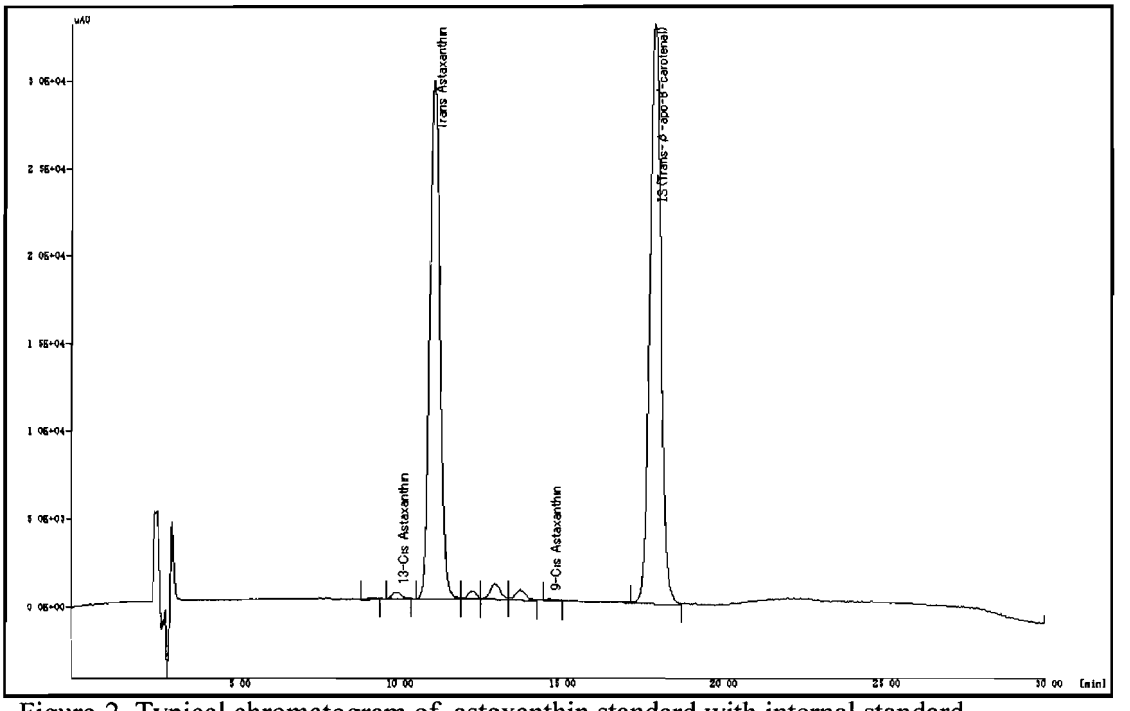


Figure-2. Typical chromatogram of astaxanthin standard with internal standard.

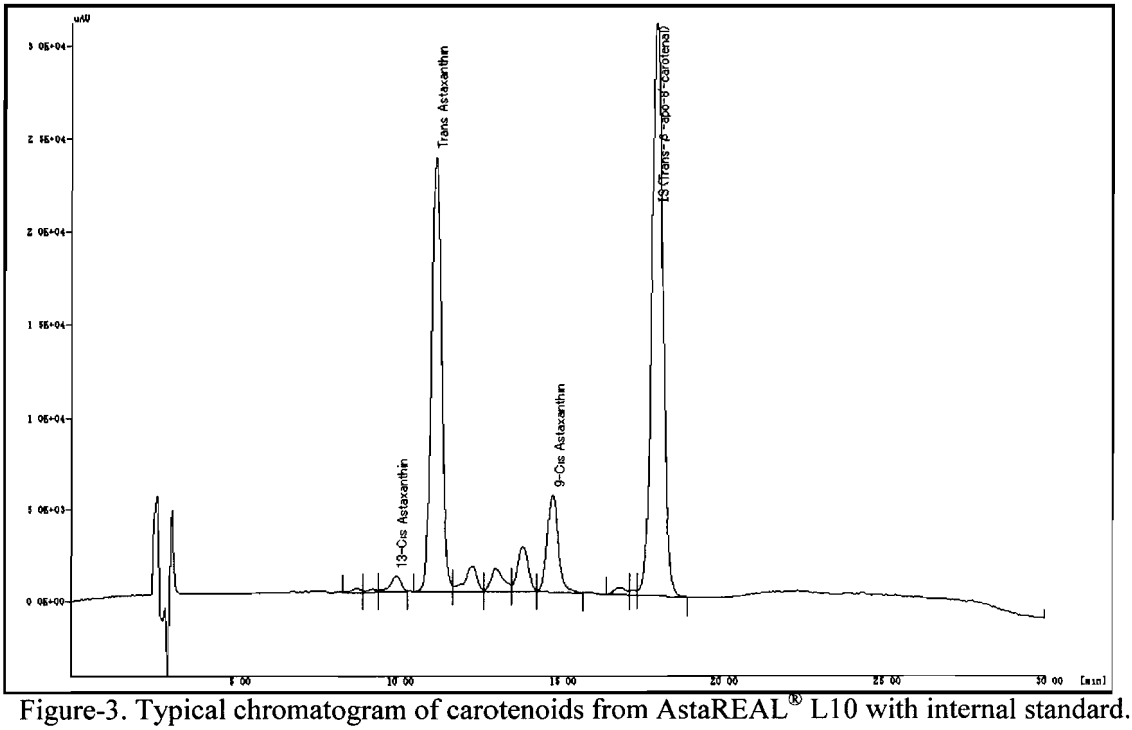


Figure-3. Typical chromatogram of carotenoids from AstaREAL® L10 with internal standard.

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**Appendix B.**

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HEALTH SCIENCES INTERNATIONAL

# ESTIMATED DAILY INTAKE OF ASTAXANTHIN BY THE U.S. POPULATION FROM PROPOSED FOOD-USES

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# ESTIMATED DAILY INTAKE OF ASTAXANTHIN BY THE U.S. POPULATION FROM PROPOSED FOOD-USES

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# ESTIMATED DAILY INTAKE OF ASTAXANTHIN BY THE U.S. POPULATION FROM PROPOSED FOOD-USES

## 1.0 INTRODUCTION

Cantox Health Sciences International has completed an assessment of the consumption of astaxanthin by the United States (U.S.) population. It is proposed for use in crackers (sweet and savory), cookies, yeast breads and rolls, bottled, enhanced, and carbonated water, carbonated beverages, energy, sports, and isotonic drinks, non-milk based meal replacements, cereal and energy bars, instant and hot cereals, ready-to-eat cereals, chewing gum, coffee (instant and regular), tea (instant, regular, and specialty), imitation and soy milk, frozen yogurt, ice cream and frozen milk desserts, hard candy, dry milks, flavored milk and milk drinks, milk-based meal replacements (ready-to-drink), yogurt, fruit drinks and ades, vegetable juices, and chocolate, confections, and other softy candy.

Estimates for the intake of astaxanthin were based on the proposed food-uses and use-levels in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2003-2004 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2009). Calculations for the mean and 90$^{th}$ percentile all-person and all-user intakes, and percentage of consumers were performed for each of the individual proposed food-uses of astaxanthin. Similar calculations were used to determine the estimated total intake of astaxanthin from all proposed food-uses combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

infants, ages 0 to 2;
children, ages 3 to 11;
female teenagers, ages 12 to 19;
male teenagers, ages 12 to 19;
female adults, ages 20 and up;
male adults, ages 20 and up; and
total population (all age and gender groups combined).

# 2.0 FOOD CONSUMPTION SURVEY DATA

## 2.1 Survey Description

NHANES for the years 2003-2004 are available for public use. NHANES are conducted on a continual yearly basis; data from these surveys are released in 2-year cycles. Each year about 7,000 people from 15 different locations across the U.S. are interviewed, and approximately 5,000 complete the health examination component of the survey. Any combination of consecutive years of data collection is a nationally representative sample of the U.S. population. It is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys, such as the typical 1-day dietary survey, overestimate consumption over longer time periods (Anderson, 1988). Because two 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) are available from the NHANES 2003-2004 survey, these data were used to generate estimates for the current intake analysis.

The surveys provide the most appropriate data for evaluating food-use and food consumption patterns in the United States and contain 2 years of data on individuals selected *via* stratified multistage probability sampling of the U.S. civilian non-institutionalized population.

NHANES 2003-2004 survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person, and Day 2 data were collected by telephone within the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S. Small counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. Fifteen PSUs are visited each year. For NHANES 2003-2004, 12,761 individuals were selected for the sample, and 10,122 were interviewed (79.3%).

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2003-2004 collected socioeconomic, physiological and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Sample weights were incorporated with NHANES 2003-2004 to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2006; USDA, 2009).

### 2.2 Statistical Methods

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of astaxanthin by the U.S. population. Estimates for the daily intake of astaxanthin represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2003-2004 data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. All-person intake refers to the estimated intake of astaxanthin averaged over all individuals surveyed, regardless of whether they consumed food products containing astaxanthin, and therefore includes "zero" consumers (those who reported no intake of food products containing astaxanthin during the 2 survey days). All-user intake refers to the estimated intake of astaxanthin by those individuals consuming food products containing astaxanthin, hence the "all-user" designation. Individuals were considered users if they consumed 1 or more food products containing astaxanthin on either Day 1 or Day 2 of the survey.

### 2.3 Statistical Reliability

Mean or percentile intake estimates based on small sample sizes or with high variability relative to the mean [assessed using the coefficient of variation (CV)] may be less statistically reliable than estimates based on adequate sample sizes or low variability relative to the mean (LSRO, 1995). Data presented herein for the estimated daily intake of astaxanthin follow the guidelines proposed by the Human Nutrition Information Service/National Center for Health Statistics Analytic Working Group for evaluating the reliability of statistical estimates adopted in the "Third Report on Nutrition Monitoring in the United States", whereby an estimated mean may be unreliable if the CV is equal to or greater than 30% (LSRO, 1995). The CV is the ratio of the estimated standard error of the mean to the estimated mean, expressed as a percentage (LSRO, 1995). Therefore, for the estimated intakes of astaxanthin presented herein, values were considered statistically unreliable if the CV was equal to or greater than 30%. These values were not considered when assessing the relative contribution of specific food-uses to total astaxanthin consumption and are marked with an asterisk. In addition, data pertaining to food uses for which fewer than 30 consumers were identified were excluded from the analysis and also are marked with an asterisk.

## 3.0 FOOD USAGE DATA

The individual proposed food-uses and use-levels for astaxanthin employed in the current intake analysis are summarized in Table 3-1. Food codes representative of each proposed food-use were chosen from the NHANES 2003-2004 (CDC, 2006; USDA, 2009). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal

Regulations (CFR, 2008a). Product-specific adjustment factors were developed based on data provided in the standard recipe file for the CSFII 1994-1996, 1998 survey (USDA, 2000). All food codes included in the current intake assessment are listed in Appendix C.

**Table 3-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Astaxanthin in the United States**

| Food Category | Proposed Food-Use | Use-Level (mg/RACC*) | RACC (g) | Use-Level (%) |
|---|---|---|---|---|
| Baked Goods and Baking Mixes | Cookies | 0.5 | 30<br>40 (brownie) | 0.0017<br>0.0013 |
| | Crackers, Savory | 0.5 | 15 (melba)<br>30 | 0.0033<br>0.0017 |
| | Crackers, Sweet | 0.5 | 30 | 0.0017 |
| | Yeast Breads and Rolls | 0 5 | 50 | 0.001 |
| Beverages and Beverage Bases | Bottled, Enhanced and Carbonated Water | 0 5 | 240 | 0.0002 |
| | Carbonated Beverages | 0.5 | 240 | 0.0002 |
| | Energy, Sports and Isotonic Drinks | 0.5 | 240 | 0.0002 |
| | Non-Milk Based Meal Replacements | 0.5 | 240 | 0.0002 |
| Cereals and Cereal Products | Cereal and Energy Bars | 0.5 | 40 | 0.0013 |
| | Instant and Hot Cereals | 0.5 | 240 (prepared) | 0.0002 |
| | Ready-to-Eat Cereals | 0.5 | 15<br>30<br>55 | 0.0033<br>0.0017<br>0.0009 |
| Chewing Gum | Chewing Gum | 0.5 | 3 | 0.0167 |
| Coffee and Tea | Coffee (Instant and Regular) | 0.5 | 240 | 0.0002 |
| | Tea (Instant, Regular and Specialty) | 0.5 | 240 | 0.0002 |
| Dairy Product Analogs | Imitation and Soy Milk | 0.5 | 240 | 0.0002 |
| Frozen Dairy Desserts and Mixes | Ice Cream and Frozen Milk Desserts | 0.5 | 120 | 0.0004 |
| | Frozen Yogurt | 0.5 | 120 | 0.0004 |
| Hard Candy | Hard Candy | 0.5 | 2 (mints)<br>15 | 0.025<br>0.0033 |
| Milk Products | Dry Milks | 0.5 | 30 | 0.0017 |
| | Flavored Milk and Milk Drinks | 0.5 | 240 | 0.0002 |
| | Milk-Based Meal Replacements, Ready-to-Drink | 0.5 | 240 | 0.0002 |
| | Yogurt | 0.5 | 225 | 0.0002 |
| Processed Fruits and Fruit Juices | Fruit Drinks and Ades | 0.5 | 240 | 0.0002 |
| Processed Vegetables and Vegetable Juices | Vegetable Juices | 0.5 | 240 | 0.0002 |
| Soft Candy | Chocolate Confections and Other Soft Candy | 0.5 | 40 | 0.0013 |

* RACC = Reference Amounts Customarily Consumed per Eating Occasion (21 CFR §101.12 - CFR, 2008b).

# 4.0 FOOD SURVEY RESULTS

Estimates for the total daily intakes of astaxanthin from all proposed food-uses are provided in Tables 4.1-1 and 4.1-2. Estimates for the daily intake of astaxanthin from individual proposed food-uses in the U.S. are summarized in Tables A-1 to A-7 and B-1 to B-7 of Appendices A and B, respectively. Tables A-1 to A-7 provide estimates for the daily intake of astaxanthin per person (mg/person/day), whereas Tables B-1 to B-7 provide estimates for the daily intake of astaxanthin on a per kilogram body weight basis (µg/kg body weight/day).

## 4.1 Estimated Daily Intake of Astaxanthin from All Proposed Food-Uses

The estimated total intake of astaxanthin from all proposed food-uses in the U.S. by population group is summarized in Table 4.1-1. Table 4.1-2 presents these data on a per kilogram body weight basis.

Approximately 97.1% of the total U.S. population were identified as potential consumers of astaxanthin from the proposed food-uses (8,030 actual users identified). Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates, and as a consequence, only the all-user intake results will be discussed in detail. Consumption of these proposed types of foods by the total U.S. population resulted in estimated mean all-user intakes of astaxanthin of 3.2 mg/person/day (52 µg/kg body weight/day) (Tables 4.1-1 and 4.1-2). The 90$^{th}$ percentile all-user intakes of astaxanthin from all proposed food-uses by the total population were 5.4 mg/person/day (93 µg/kg body weight/day).

The population groups that contained the greatest percentage of users were children, female teenagers, and male teenagers of which 100% reported consuming the foods in which astaxanthin is proposed for use. The lowest percentage of users of any population group was reported for infants, at 75.3%. On an individual population basis, the greatest estimated mean all-user intake of astaxanthin on an absolute basis was observed to occur in male adults at 3.8 mg/person/day. Infants displayed the lowest estimated mean all-user intake of astaxanthin on an absolute basis with a value of 1.1 mg/person/day. On a body weight basis, the mean all-user intake of astaxanthin was estimated to be highest in infants with a value of 91 µg/kg body weight/day, while female adults displayed the lowest all-user mean intake on a body weight basis at 43 µg/kg body weight/day.

**Table 4.1-1 Summary of the Estimated Daily Intake of Astaxanthin from All Proposed Food-Uses in the U.S. by Population Group (2003-2004 NHANES Data)**

| Population Group | Age Group (Years) | % Users | Actual # of Total Users | All-Person Consumption | | All-User Consumption | |
|---|---|---|---|---|---|---|---|
| | | | | Mean (mg) | 90th Percentile (mg) | Mean (mg) | 90th Percentile (mg) |
| Infants | 0 to 2 | 75.3 | 700 | 0.9 | 2.1 | 1.1 | 2.3 |
| Children | 3 to 11 | 100 | 1,287 | 2.4 | 3.9 | 2.4 | 3.9 |
| Female Teenagers | 12 to 19 | 100 | 992 | 2.6 | 4.3 | 2.6 | 4.3 |
| Male Teenagers | 12 to 19 | 100 | 999 | 3.4 | 5.4 | 3.4 | 5.4 |
| Female Adults | 20 and Up | 99.9 | 2,126 | 3.0 | 5.0 | 3.0 | 5.0 |
| Male Adults | 20 and Up | 99.8 | 1,926 | 3.8 | 6.3 | 3.8 | 6.3 |
| Total Population | All Ages | 97.1 | 8,030 | 3.1 | 5.4 | 3.2 | 5.4 |

When heavy consumers (90th percentile) were assessed, the all-user intake of astaxanthin from all proposed food-uses also was determined to be greatest in male adults at 6.3 mg/person/day. The lowest 90th percentile all-user intake was observed to occur in infants with a value of 2.3 mg/person/day. On a body weight basis, infants were again observed to display the largest all-user 90th percentile intake of astaxanthin with a value of 167 µg/kg body weight/day. The lowest all-user 90th percentile intake of astaxanthin on a body weight basis was again observed to occur in female adults, with an estimated astaxanthin consumption of 74 µg/kg body weight/day.

**Table 4.1-2 Summary of the Estimated Daily per Kilogram Body Weight Intake of Astaxanthin from All Proposed Food-Uses in the U.S. by Population Group (2003-2004 NHANES Data)**

| Population Group | Age Group (Years) | % Users | Actual # of Total Users | All-Person Consumption (µg/kg body weight) | | All-User Consumption (µg/kg body weight) | |
|---|---|---|---|---|---|---|---|
| | | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Infants | 0 to 2 | 75.3 | 700 | 76 | 155 | 91 | 167 |
| Children | 3 to 11 | 100 | 1,287 | 90 | 150 | 90 | 150 |
| Female Teenagers | 12 to 19 | 100 | 992 | 45 | 76 | 45 | 76 |
| Male Teenagers | 12 to 19 | 100 | 999 | 53 | 94 | 53 | 94 |
| Female Adults | 20 and Up | 99.9 | 2,126 | 43 | 74 | 43 | 74 |
| Male Adults | 20 and Up | 99.8 | 1,926 | 45 | 75 | 45 | 75 |
| Total Population | All Ages | 97.1 | 8,030 | 52 | 93 | 52 | 93 |

### 4.1.1 All-Person Intakes

Estimates for the mean and 90th percentile daily intakes of astaxanthin from each individual proposed food-use are summarized in Tables A-1 to A-7 and B-1 to B-7 on a mg/person/day and µg/kg body weight/day basis, respectively. Tables A-7 and B-7 summarize the estimates for the mean all-person intakes of astaxanthin by the total population (all ages) from each of the individual proposed food-uses on a mg/person/day and µg/kg body weight/day basis, respectively. The total U.S. population was identified as being significant consumers of yeast breads and rolls (78.5% users), carbonated beverages (63.8% users), and ready-to-eat cereals (40.6% users).

Consumption of carbonated beverages provided the largest mean and 90th percentile all-person intakes of astaxanthin for the total U.S. population, at 0.8 mg/person/day (12 µg/kg body weight/day) and 2.1 mg/person/day (30 µg/kg body weight/day), respectively. In addition, high mean and 90th percentile all-person intakes of astaxanthin resulted from the consumption of coffee (instant and regular) (0.5 and 1.4 mg/person/day, respectively), yeast breads and rolls (0.5 and 1.0 mg/person/day, respectively) and tea (instant, regular and specialty) (0.3 and 0.9 mg/person/day, respectively). On a body weight basis, mean and 90th percentile all-person intakes for coffee (instant and regular) were estimated to be 6 and 18 µg/kg body weight/day, for yeast breads and rolls were 8 and 17 µg/kg body weight/day, and for tea (instant, regular and specialty) were 4 and 13 µg/kg body weight/day.

Of the individual population groups, the highest mean and 90th percentile all-person intakes of astaxanthin were determined to result from the consumption of fruit drinks and ades in infants and from the consumption of carbonated beverages in all other population groups (Tables A-1 to A-6 and Tables B-1 to B-6). The highest mean all-person intakes of astaxanthin were reported in male teenagers and male adults consuming carbonated beverages, at 1.1 mg/person/day (17 and 13 µg/kg body weight/day for male teenagers and male adults, respectively). The highest 90th percentile all-person intake of astaxanthin was reported in male adults consuming carbonated beverages, at 2.6 mg/person/ day (30 µg/kg body weight/day). On a body weight basis, consumption of carbonated beverages by male teenagers led to the highest mean and 90th percentile all-person intakes of astaxanthin at 17 and 36 µg/kg body weight/day, respectively.

### 4.1.2 All-User Intakes

Tables A-7 and B-7 also summarize the estimates for the mean and 90th percentile all-user intakes of astaxanthin by the total population (all ages) from each of the individual food-uses on a mg/person/day and µg/kg body weight/day basis, respectively. For all-user intakes, the contribution of each food-use to the overall intake is a function of both the estimated intake of astaxanthin resulting from the consumption of the food, as well as the percentage of users identified as consumers of the food. For example, for the total population, the consumption of

energy, sports, and isotonic drinks resulted in an estimated mean all-user astaxanthin intake of 0.8 mg/person/day; however, only 476 users (5.8% of the total population) of energy, sports, and isotonic drinks were identified. Therefore, the contribution of this food-use to the mean all-user intake of astaxanthin was not as important as the contribution of yeast breads and rolls, for which the mean all-user intake of astaxanthin was lower at 0.6 mg/person/day but the number of users was greater at 6,489 (78.5% of the total population).

Consumers of carbonated beverages were identified as making the greatest contribution to the mean and 90$^{th}$ percentile all-user intakes of astaxanthin for the total U.S. population at 1.2 and 2.5 mg/person/day, respectively. Of the other food-uses, the consumption of yeast breads and rolls, coffee (instant and regular), and fruit drinks and ades also made significant contributions to the estimates for the mean (0.6, 1.0, and 0.6 mg/person/day, respectively) and 90$^{th}$ percentile (1.1, 2.0, and 1.4 mg/person/day, respectively) all-user intakes of astaxanthin by the total population.

On an individual population group basis, the most significant contribution to the mean and 90$^{th}$ percentile all-user intakes of astaxanthin resulted from the consumption of fruit drinks and ades by infants, and the consumption of carbonated beverages by all other population groups (Tables A-1 to A-6 and Tables B-1 to B-6). Male teenagers consuming carbonated beverages made the largest contribution to the mean all-user intake of astaxanthin at 1.3 mg/person/day (20 µg/kg body weight/day). Male adults consuming carbonated beverages made the largest contribution to the 90$^{th}$ percentile all-user intake of astaxanthin at 3.1 mg/person/day (35 µg/kg body weight/day). On a per kilogram body weight basis, infants consuming energy, sports, and isotonic drinks experienced the highest mean and 90$^{th}$ percentile all-user intakes of astaxanthin at 48 and 103 µg/kg body weight/day.

The estimated intakes of astaxanthin were considered statistically unreliable if the CV was equal to or greater than 30%, and when the total number of users for a given food use was less than 30. Assessing the CV and number of users for all-user intake estimates found the intake values for bottled, enhanced, and carbonated water, non-milk based meal replacements, imitation and soy milk, frozen yogurt, and milk-based meal replacement beverages (ready-to-drink) to be statistically unreliable in the infant, children, female teenager, and male teenager population groups. The all-user intake estimates for cereal and energy bars, chewing gum, and coffee (instant and regular) were statistically unreliable in the infant population group. Instant and hot cereals and vegetable juice estimated values were statistically unreliable in the female teenager and male teenager population groups. The estimated intakes for dry milks were statistically unreliable in the female adult and male adult population groups, and the estimated intake values for non-milk based meal replacements were statistically unreliable in the female adult population group.

## 5.0 CONCLUSIONS

Consumption data and information pertaining to the individual proposed food-uses of astaxanthin were used to estimate the all-person and all-user intakes of astaxanthin for specific demographic groups and for the total U.S. population. This type of intake methodology is generally considered to be 'worst case' as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, overestimate the consumption of food products that are consumed relatively infrequently.

In summary, on an all-user basis, the mean intake of astaxanthin by the total U.S. population from all proposed food-uses was estimated to be 3.2 mg/person/day or 52 µg/kg body weight/day. The heavy consumer (90$^{th}$ percentile) all-user intake of astaxanthin by the total U.S. population from all proposed food-uses was estimated to be 5.4 mg/person/day or 93 µg/kg body weight/day. The most significant contribution to the mean and 90$^{th}$ percentile all-user intakes of astaxanthin resulted from the consumption of fruit drinks and ades by infants, and the consumption of carbonated beverages by all other population groups.

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## APPENDIX A

## Estimated Daily Intake of Astaxanthin from Individual Proposed Food-Uses by Different Population Groups Within the United States

**Table A-1 Estimated Daily Intake of Astaxanthin by Infants (Aged 0 to 2 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption | | All-Users Consumption | |
|---|---|---|---|---|---|---|
| | | | Mean (mg) | 90th Percentile (mg) | Mean (mg) | 90th Percentile (mg) |
| Baked Goods and Baking Mixes | | | | | | |
| Cookies | 39.9 | 371 | 0.1 | 0.3 | 0.2 | 0.4 |
| Crackers, Savory | 30.4 | 283 | 0.1 | 0.2 | 0.2 | 0.4 |
| Crackers, Sweet | 17.3 | 161 | < 0.1 | 0.2 | 0.2 | 0.4 |
| Yeast Breads and Rolls | 41.9 | 390 | 0.1 | 0.4 | 0.2 | 0.5 |
| Beverages and Beverage Bases | | | | | | |
| Bottled, Enhanced, and Carbonated Water | 0.6 | 6 | < 0.1* | na | 0.1* | 0.1* |
| Carbonated Beverages | 22.3 | 207 | 0.1 | 0.2 | 0.2 | 0.6 |
| Energy, Sports, and Isotonic Drinks | 4.7 | 44 | < 0.1 | na | 0.6 | 1.2 |
| Non-Milk Based Meal Replacements | 0.2 | 2 | < 0.1* | na | 0.3* | 0.3* |
| Cereals and Cereal Products | | | | | | |
| Cereal and Energy Bars | 2.3 | 21 | < 0.1* | na | 0.2* | 0.3* |
| Instant and Hot Cereals | 11.8 | 110 | < 0.1 | < 0.1 | 0.2 | 0.4 |
| Ready-to-Eat Cereals | 41.4 | 385 | 0.1 | 0.4 | 0.3 | 0.5 |
| Chewing Gum | | | | | | |
| Chewing Gum | 1.4 | 13 | < 0.1* | na | 0.4* | 0.7* |
| Coffee and Tea | | | | | | |
| Coffee (Instant and Regular) | 1.2 | 11 | < 0.1* | na | 0.1* | 0.3* |
| Tea (Instant, Regular, and Specialty) | 5.6 | 52 | < 0.1 | na | 0.4 | 0.6 |
| Dairy Product Analogs | | | | | | |
| Imitation and Soy Milk | 0.5 | 5 | < 0.1* | na | 0.6* | 1.8* |

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**Table A-1 Estimated Daily Intake of Astaxanthin by Infants (Aged 0 to 2 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption | | All-Users Consumption | |
|---|---|---|---|---|---|---|
| | | | Mean (mg) | 90th Percentile (mg) | Mean (mg) | 90th Percentile (mg) |
| Frozen Dairy Desserts and Mixes | | | | | | |
| Frozen Yogurt | 1.0 | 9 | < 0.1* | na | 0.2* | 0.2* |
| Ice Cream and Frozen Milk Desserts | 15.1 | 140 | < 0.1 | 0.1 | 0.1 | 0.3 |
| Hard Candy | | | | | | |
| Hard Candy | 8.8 | 82 | < 0.1 | na | 0.2 | 0.3 |
| Milk Products | | | | | | |
| Dry Milks | 5.9 | 55 | < 0.1 | na | < 0.1 | 0.1 |
| Flavored Milk and Milk Drinks | 9.7 | 90 | < 0.1 | 0.1 | 0.4 | 1.0 |
| Milk-Based Meal Replacements, Ready-to-Drink | 0.1 | 1 | < 0.1* | na | 0.1* | 0.1* |
| Yogurt | 13.0 | 121 | < 0.1 | 0.1 | 0.1 | 0.2 |
| Processed Fruits and Fruit Juices | | | | | | |
| Fruit Drinks and Ades | 29.2 | 272 | 0.1 | 0.5 | 0.5 | 1.0 |
| Processed Vegetables and Vegetable Juices | | | | | | |
| Vegetable Juices | 6.1 | 57 | < 0.1 | na | 0.1 | 0.2 |
| Soft Candy | | | | | | |
| Chocolate Confections and Other Soft Candy | 18.8 | 175 | < 0.1 | 0.1 | 0.2 | 0.4 |

na = not applicable
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% or the sample size is less than 30 (see Section 2.3).

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**Table A-2 Estimated Daily Intake of Astaxanthin by Children (Aged 3 to 11 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption | | All-Users Consumption | |
|---|---|---|---|---|---|---|
| | | | Mean (mg) | 90th Percentile (mg) | Mean (mg) | 90th Percentile (mg) |
| Baked Goods and Baking Mixes | | | | | | |
| Cookies | 49.8 | 641 | 0.2 | 0.5 | 0.4 | 0.7 |
| Crackers, Savory | 25.8 | 332 | 0.1 | 0.3 | 0.2 | 0.5 |
| Crackers, Sweet | 11.7 | 151 | < 0.1 | 0.1 | 0.2 | 0.5 |
| Yeast Breads and Rolls | 83.5 | 1,075 | 0.4 | 0.8 | 0.5 | 0.9 |
| Beverages and Beverage Bases | | | | | | |
| Bottled, Enhanced, and Carbonated Water | 0.9 | 12 | < 0.1* | na | 0.6* | 2.0* |
| Carbonated Beverages | 63.1 | 812 | 0.4 | 1.0 | 0.6 | 1.2 |
| Energy, Sports, and Isotonic Drinks | 7.5 | 97 | < 0.1 | na | 0.5 | 0.7 |
| Non-Milk Based Meal Replacements | 0.4 | 5 | < 0.1* | na | 0.3* | 0.3* |
| Cereals and Cereal Products | | | | | | |
| Cereal and Energy Bars | 4.1 | 53 | < 0.1 | na | 0.3 | 0.6 |
| Instant and Hot Cereals | 7.4 | 95 | < 0.1 | na | 0.3 | 0.5 |
| Ready-to-Eat Cereals | 66.0 | 849 | 0.3 | 0.9 | 0.5 | 1.0 |
| Chewing Gum | | | | | | |
| Chewing Gum | 6.4 | 82 | < 0.1 | na | 0.5 | 1.0 |
| Coffee and Tea | | | | | | |
| Coffee (Instant and Regular) | 2.8 | 36 | < 0.1 | na | 0.2 | 0.5 |
| Tea (Instant, Regular, and Specialty) | 13.1 | 168 | 0.1 | 0.3 | 0.5 | 1.3 |
| Dairy Product Analogs | | | | | | |
| Imitation and Soy Milk | 0.5 | 7 | < 0.1* | na | 0.5* | 1.5* |

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**Table A-2 Estimated Daily Intake of Astaxanthin by Children (Aged 3 to 11 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption | | All-Users Consumption | |
|---|---|---|---|---|---|---|
| | | | Mean (mg) | 90th Percentile (mg) | Mean (mg) | 90th Percentile (mg) |
| Frozen Dairy Desserts and Mixes | | | | | | |
| Frozen Yogurt | 1.3 | 17 | < 0.1* | na | 0.2* | 0.4* |
| Ice Cream and Frozen Milk Desserts | 33.8 | 435 | 0.1 | 0.3 | 0.2 | 0.4 |
| Hard Candy | | | | | | |
| Hard Candy | 18.0 | 232 | 0.1 | 0.2 | 0.4 | 0.8 |
| Milk Products | | | | | | |
| Dry Milks | 11.0 | 142 | < 0.1 | < 0.1 | 0.1 | 0.2 |
| Flavored Milk and Milk Drinks | 34.0 | 438 | 0.1 | 0.5 | 0.4 | 0.7 |
| Milk-Based Meal Replacements, Ready-to-Drink | 0.3 | 4 | < 0.1* | na | 0.4* | 1.2* |
| Yogurt | 12.8 | 165 | < 0.1 | 0.1 | 0.2 | 0.2 |
| Processed Fruits and Fruit Juices | | | | | | |
| Fruit Drinks and Ades | 61.6 | 793 | 0.3 | 0.8 | 0.5 | 1.0 |
| Processed Vegetables and Vegetable Juices | | | | | | |
| Vegetable Juices | 2.9 | 37 | < 0.1 | na | 0.1 | 0.2 |
| Soft Candy | | | | | | |
| Chocolate Confections and Other Soft Candy | 40.6 | 522 | 0.1 | 0.4 | 0.3 | 0.5 |

na = not applicable
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% or the sample size is less than 30 (see Section 2.3).

**Table A-3 Estimated Daily Intake of Astaxanthin by Female Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption | | All-Users Consumption | |
|---|---|---|---|---|---|---|
| | | | Mean (mg) | 90th Percentile (mg) | Mean (mg) | 90th Percentile (mg) |
| Baked Goods and Baking Mixes | | | | | | |
| Cookies | 35.8 | 355 | 0.1 | 0.4 | 0.3 | 0.7 |
| Crackers, Savory | 16.2 | 161 | < 0.1 | 0.2 | 0.3 | 0.6 |
| Crackers, Sweet | 4.9 | 49 | < 0.1 | na | 0.3 | 0.5 |
| Yeast Breads and Rolls | 78.6 | 780 | 0.4 | 0.9 | 0.5 | 1.0 |
| Beverages and Beverage Bases | | | | | | |
| Bottled, Enhanced, and Carbonated Water | 1.9 | 19 | < 0.1* | na | 0.3* | 0.5* |
| Carbonated Beverages | 75.6 | 750 | 0.9 | 1.9 | 1.1 | 2.1 |
| Energy, Sports, and Isotonic Drinks | 6.5 | 64 | < 0.1 | na | 0.5 | 0.7 |
| Non-Milk Based Meal Replacements | 0.3 | 3 | < 0.1* | na | 0.2* | 0.4* |
| Cereal and Cereal Products | | | | | | |
| Cereal and Energy Bars | 6.0 | 60 | < 0.1 | na | 0.4 | 0.5 |
| Instant and Hot Cereals | 2.7 | 27 | < 0.1* | na | 0.3* | 0.5* |
| Ready-to-Eat Cereals | 41.7 | 414 | 0.3 | 0.7 | 0.6 | 1.1 |
| Chewing Gum | | | | | | |
| Chewing Gum | 9.8 | 97 | 0.1 | na | 0.8 | 2.3 |
| Coffee and Tea | | | | | | |
| Coffee (Instant and Regular) | 10.8 | 107 | 0.1 | 0.2 | 0.5 | 0.9 |
| Tea (Instant, Regular, and Specialty) | 21.4 | 212 | 0.1 | 0.6 | 0.6 | 1.2 |
| Dairy Product Analogs | | | | | | |
| Imitation and Soy Milk | 0.7 | 7 | < 0.1* | na | 0.6* | 0.7* |

**Table A-3 Estimated Daily Intake of Astaxanthin by Female Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption | | All-Users Consumption | |
|---|---|---|---|---|---|---|
| | | | Mean (mg) | 90th Percentile (mg) | Mean (mg) | 90th Percentile (mg) |
| Frozen Dairy Desserts and Mixes | | | | | | |
| Frozen Yogurt | 1.1 | 11 | < 0.1* | na | 0.3* | 0.6* |
| Ice Cream and Frozen Milk Desserts | 25.5 | 253 | 0.1 | 0.3 | 0.3 | 0.5 |
| Hard Candy | | | | | | |
| Hard Candy | 12.6 | 125 | < 0.1 | < 0.1 | 0.3 | 0.7 |
| Milk Products | | | | | | |
| Dry Milks | 9.6 | 95 | < 0.1 | < 0.1 | < 0.1 | 0.2 |
| Flavored Milk and Milk Drinks | 18.6 | 185 | 0.1 | 0.3 | 0.4 | 0.7 |
| Milk-Based Meal Replacements, Ready-to-Drink | 0.8 | 8 | < 0.1* | na | 0.5 | 0.7 |
| Yogurt | 7.0 | 69 | < 0.1 | na | 0.2 | 0.4 |
| Processed Fruits and Fruit Juices | | | | | | |
| Fruit Drinks and Ades | 49.2 | 488 | 0.2 | 0.7 | 0.6 | 1.3 |
| Processed Vegetables and Vegetable Juices | | | | | | |
| Vegetable Juices | 1.6 | 16 | < 0.1* | na | 0.2* | 0.6* |
| Soft Candy | | | | | | |
| Chocolate Confections and Other Soft Candy | 41.2 | 409 | 0.1 | 0.5 | 0.3 | 0.7 |

na = not applicable
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% or the sample size is less than 30 (see Section 2.3).

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**Table A-4 Estimated Daily Intake of Astaxanthin by Male Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption | | All-Users Consumption | |
|---|---|---|---|---|---|---|
| | | | Mean (mg) | 90th Percentile (mg) | Mean (mg) | 90th Percentile (mg) |
| Baked Goods and Baking Mixes | | | | | | |
| Cookies | 34.3 | 343 | 0.2 | 0.5 | 0.4 | 0.8 |
| Crackers, Savory | 12.1 | 121 | < 0.1 | 0.1 | 0.4 | 0.9 |
| Crackers, Sweet | 3.9 | 39 | < 0.1 | na | 0.2 | 0.5 |
| Yeast Breads and Rolls | 83.0 | 829 | 0.6 | 1.2 | 0.7 | 1.3 |
| Beverages and Beverage Bases | | | | | | |
| Bottled, Enhanced, and Carbonated Water | 2.2 | 22 | < 0.1* | na | 0.4* | 0.5* |
| Carbonated Beverages | 81.0 | 809 | 1.1 | 2.4 | 1.3 | 2.6 |
| Energy, Sports, and Isotonic Drinks | 11.9 | 119 | 0.1 | 0.5 | 0.9 | 1.9 |
| Non-Milk Based Meal Replacements | 0.3 | 3 | < 0.1* | na | 0.3* | 0.3* |
| Cereals and Cereal Products | | | | | | |
| Cereal and Energy Bars | 4.2 | 42 | < 0.1 | na | 0.4 | 0.7 |
| Instant and Hot Cereals | 2.0 | 20 | < 0.1* | na | 0.2* | 0.4* |
| Ready-to-Eat Cereals | 43.6 | 436 | 0.3 | 1.0 | 0.8 | 1.4 |
| Chewing Gum | | | | | | |
| Chewing Gum | 8.5 | 85 | < 0.1 | na | 0.6 | 1.0 |
| Coffee and Tea | | | | | | |
| Coffee (Instant and Regular) | 6.8 | 68 | < 0.1 | na | 0.8 | 2.8 |
| Tea (Instant, Regular, and Specialty) | 18.4 | 184 | 0.2 | 0.6 | 0.7 | 1.4 |
| Dairy Product Analogs | | | | | | |
| Imitation and Soy Milk | 0.3 | 3 | < 0.1* | na | 0.3* | 0.4* |

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**Table A-4 Estimated Daily Intake of Astaxanthin by Male Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption | | All-Users Consumption | |
|---|---|---|---|---|---|---|
| | | | Mean (mg) | 90th Percentile (mg) | Mean (mg) | 90th Percentile (mg) |
| Frozen Dairy Desserts and Mixes | | | | | | |
| Frozen Yogurt | 0.4 | 4 | < 0.1* | na | 0.7* | 1.5* |
| Ice Cream and Frozen Milk Desserts | 23.3 | 233 | 0.1 | 0.3 | 0.3 | 0.6 |
| Hard Candy | | | | | | |
| Hard Candy | 9.4 | 94 | < 0.1 | < 0.1 | 0.3 | 0.7 |
| Milk Products | | | | | | |
| Dry Milks | 7.0 | 70 | < 0.1 | na | 0.1 | 0.2 |
| Flavored Milk and Milk Drinks | 19.3 | 193 | 0.1 | 0.3 | 0.4 | 0.9 |
| Milk-Based Meal Replacements, Ready-to-Drink | 0.7 | 7 | < 0.1* | na | 0.5* | 0.6* |
| Yogurt | 3.2 | 32 | < 0.1 | na | 0.2 | 0.4 |
| Processed Fruits and Fruit Juices | | | | | | |
| Fruit Drinks and Ades | 48.1 | 481 | 0.3 | 1.2 | 0.9 | 1.8 |
| Processed Vegetables and Vegetable Juices | | | | | | |
| Vegetable Juices | 1.5 | 15 | < 0.1* | na | 0.6* | 1.0* |
| Soft Candy | | | | | | |
| Chocolate Confections and Other Soft Candy | 32.9 | 329 | 0.1 | 0.5 | 0.5 | 1.0 |

na = not applicable
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% or the sample size is less than 30 (see Section 2.3).

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**Table A-5 Estimated Daily Intake of Astaxanthin by Female Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption | | All-Users Consumption | |
|---|---|---|---|---|---|---|
| | | | Mean (mg) | 90th Percentile (mg) | Mean (mg) | 90th Percentile (mg) |
| Baked Goods and Baking Mixes | | | | | | |
| Cookies | 34.6 | 737 | 0.1 | 0.4 | 0.4 | 0.7 |
| Crackers, Savory | 24.1 | 514 | 0.1 | 0.2 | 0.2 | 0.5 |
| Crackers, Sweet | 7.1 | 151 | < 0.1 | na | 0.2 | 0.6 |
| Yeast Breads and Rolls | 82.6 | 1,758 | 0.4 | 0.9 | 0.5 | 0.9 |
| Beverages and Beverage Bases | | | | | | |
| Bottled, Enhanced, and Carbonated Water | 2.9 | 62 | < 0.1 | na | 0.7 | 1.5 |
| Carbonated Beverages | 63.8 | 1,358 | 0.7 | 2.0 | 1.1 | 2.3 |
| Energy, Sports, and Isotonic Drinks | 1.6 | 35 | < 0.1 | na | 0.9 | 2.9 |
| Non-Milk Based Meal Replacements | 1.2 | 26 | < 0.1* | na | 0.3* | 0.5* |
| Cereals and Cereal Products | | | | | | |
| Cereal and Energy Bars | 5.9 | 126 | < 0.1 | na | 0.4 | 0.7 |
| Instant and Hot Cereals | 13.4 | 285 | < 0.1 | < 0.1 | 0.3 | 0.5 |
| Ready-to-Eat Cereals | 32.7 | 697 | 0.2 | 0.5 | 0.5 | 0.9 |
| Chewing Gum | | | | | | |
| Chewing Gum | 3.7 | 79 | < 0.1 | na | 0.6 | 1.3 |
| Coffee and Tea | | | | | | |
| Coffee (Instant and Regular) | 58.9 | 1,253 | 0.6 | 1.5 | 1.0 | 1.9 |
| Tea (Instant, Regular, and Specialty) | 35.8 | 762 | 0.3 | 1.0 | 0.9 | 1.9 |
| Dairy Product Analogs | | | | | | |
| Imitation and Soy Milk | 2.7 | 58 | < 0.1 | na | 0.4 | 1.0 |

**Table A-5 Estimated Daily Intake of Astaxanthin by Female Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption | | All-Users Consumption | |
|---|---|---|---|---|---|---|
| | | | Mean (mg) | 90th Percentile (mg) | Mean (mg) | 90th Percentile (mg) |
| Frozen Dairy Desserts and Mixes | | | | | | |
| Frozen Yogurt | 2.2 | 47 | < 0.1 | na | 0.3 | 0.8 |
| Ice Cream and Frozen Milk Desserts | 25.2 | 537 | 0.1 | 0.3 | 0.2 | 0.5 |
| Hard Candy | | | | | | |
| Hard Candy | 7.5 | 160 | < 0.1 | na | 0.5 | 0.8 |
| Milk Products | | | | | | |
| Dry Milks | 8.8 | 187 | < 0.1* | na | 0.3* | 0.3* |
| Flavored Milk and Milk Drinks | 9.2 | 196 | < 0.1 | na | 0.5 | 0.9 |
| Milk-Based Meal Replacements, Ready-to-Drink | 2.3 | 49 | < 0.1 | na | 0.6 | 1.0 |
| Yogurt | 10.1 | 216 | < 0.1 | < 0.1 | 0.2 | 0.3 |
| Processed Fruits and Fruit Juices | | | | | | |
| Fruit Drinks and Ades | 28.7 | 612 | 0.2 | 0.5 | 0.6 | 1.3 |
| Processed Vegetables and Vegetable Juices | | | | | | |
| Vegetable Juices | 5.4 | 115 | < 0.1 | na | 0.2 | 0.4 |
| Soft Candy | | | | | | |
| Chocolate Confections and Other Soft Candy | 32.5 | 691 | 0.1 | 0.3 | 0.3 | 0.6 |

na = not applicable
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% or the sample size is less than 30 (see Section 2.3).



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**Table A-6 Estimated Daily Intake of Astaxanthin by Male Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption | | All-Users Consumption | |
|---|---|---|---|---|---|---|
| | | | Mean (mg) | 90th Percentile (mg) | Mean (mg) | 90th Percentile (mg) |
| Baked Goods and Baking Mixes | | | | | | |
| Cookies | 33.2 | 640 | 0.2 | 0.5 | 0.5 | 0.9 |
| Crackers, Savory | 18.9 | 365 | 0.1 | 0.2 | 0.3 | 0.6 |
| Crackers, Sweet | 5.4 | 104 | < 0.1 | na | 0.3 | 0.5 |
| Yeast Breads and Rolls | 85.9 | 1,657 | 0.6 | 1.2 | 0.7 | 1.3 |
| Beverages and Beverage Bases | | | | | | |
| Bottled, Enhanced, and Carbonated Water | 1.9 | 36 | < 0.1 | na | 0.7 | 1.0 |
| Carbonated Beverages | 69.2 | 1,335 | 1.1 | 2.6 | 1.4 | 3.1 |
| Energy, Sports, and Isotonic Drinks | 6.1 | 117 | 0.1 | na | 0.9 | 1.8 |
| Non-Milk Based Meal Replacements | 1.9 | 37 | < 0.1 | na | 0.7 | 1.2 |
| Cereals and Cereal Products | | | | | | |
| Cereal and Energy Bars | 5.1 | 98 | < 0.1 | na | 0.4 | 0.6 |
| Instant and Hot Cereals | 10.3 | 199 | < 0.1 | na | 0.4 | 0.8 |
| Ready-to-Eat Cereals | 30.0 | 578 | 0.2 | 0.7 | 0.7 | 1.3 |
| Chewing Gum | | | | | | |
| Chewing Gum | 3.2 | 62 | < 0.1 | na | 0.5 | 0.8 |
| Coffee and Tea | | | | | | |
| Coffee (Instant and Regular) | 62.9 | 1,214 | 0.7 | 1.8 | 1.2 | 2.4 |
| Tea (Instant, Regular, and Specialty) | 29.8 | 574 | 0.4 | 1.2 | 1.2 | 2.6 |
| Dairy Product Analogs | | | | | | |
| Imitation and Soy Milk | 2.0 | 39 | < 0.1 | na | 0.4 | 0.8 |

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**Table A-6 Estimated Daily Intake of Astaxanthin by Male Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption | | All-Users Consumption | |
|---|---|---|---|---|---|---|
| | | | Mean (mg) | 90th Percentile (mg) | Mean (mg) | 90th Percentile (mg) |
| Frozen Dairy Desserts and Mixes | | | | | | |
| Frozen Yogurt | 1.9 | 36 | < 0.1 | na | 0.4 | 0.6 |
| Ice Cream and Frozen Milk Desserts | 24.0 | 463 | 0.1 | 0.3 | 0.3 | 0.6 |
| Hard Candy | | | | | | |
| Hard Candy | 6.0 | 115 | < 0.1 | na | 0.4 | 0.9 |
| Milk Products | | | | | | |
| Dry Milks | 8.3 | 160 | < 0.1* | na | 0.1* | 0.2* |
| Flavored Milk and Milk Drinks | 7.1 | 136 | < 0.1 | na | 0.5 | 1.0 |
| Milk-Based Meal Replacements, Ready-to-Drink | 1.8 | 35 | < 0.1 | na | 0.9 | 2.1 |
| Yogurt | 5.6 | 108 | < 0.1 | na | 0.2 | 0.4 |
| Processed Fruits and Fruit Juices | | | | | | |
| Fruit Drinks and Ades | 27.6 | 533 | 0.2 | 0.6 | 0.8 | 1.6 |
| Processed Vegetables and Vegetable Juices | | | | | | |
| Vegetable Juices | 4.6 | 88 | < 0.1 | na | 0.3 | 0.6 |
| Soft Candy | | | | | | |
| Chocolate Confections and Other Soft Candy | 26.0 | 502 | 0.1 | 0.4 | 0.4 | 0.7 |

na = not applicable
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% or the sample size is less than 30 (see Section 2.3).

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**Table A-7 Estimated Daily Intake of Astaxanthin from Individual Proposed Food-Uses by the Total Population (All Ages) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption | | All-Users Consumption | |
|---|---|---|---|---|---|---|
| | | | Mean (mg) | 90th Percentile (mg) | Mean (mg) | 90th Percentile (mg) |
| Baked Goods and Baking Mixes | | | | | | |
| Cookies | 37.3 | 3,087 | 0.1 | 0.5 | 0.4 | 0.8 |
| Crackers, Savory | 21.5 | 1,776 | 0.1 | 0.2 | 0.3 | 0.5 |
| Crackers, Sweet | 7.9 | 655 | < 0.1 | na | 0.2 | 0.5 |
| Yeast Breads and Rolls | 78.5 | 6,489 | 0.5 | 1.0 | 0.6 | 1.1 |
| Beverages and Beverage Bases | | | | | | |
| Bottled, Enhanced, and Carbonated Water | 1.9 | 157 | < 0.1 | na | 0.6 | 1.5 |
| Carbonated Beverages | 63.8 | 5,271 | 0.8 | 2.1 | 1.2 | 2.5 |
| Energy, Sports, and Isotonic Drinks | 5.8 | 476 | < 0.1 | na | 0.8 | 1.7 |
| Non-Milk Based Meal Replacements | 0.9 | 76 | < 0.1 | na | 0.5 | 1.0 |
| Cereals and Cereal Products | | | | | | |
| Cereal and Energy Bars | 4.8 | 400 | < 0.1 | na | 0.4 | 0.6 |
| Instant and Hot Cereals | 8.9 | 736 | < 0.1 | na | 0.3 | 0.6 |
| Ready-to-Eat Cereals | 40.6 | 3,359 | 0.2 | 0.7 | 0.6 | 1.1 |
| Chewing Gum | | | | | | |
| Chewing Gum | 5.1 | 418 | < 0.1 | na | 0.5 | 1.2 |
| Coffee and Tea | | | | | | |
| Coffee (Instant and Regular) | 32.5 | 2,689 | 0.5 | 1.4 | 1.0 | 2.0 |
| Tea (Instant, Regular, and Specialty) | 23.6 | 1,952 | 0.3 | 0.9 | 1.0 | 2.0 |
| Dairy Product Analogs | | | | | | |
| Imitation and Soy Milk | 1.4 | 119 | < 0.1 | na | 0.4 | 1.0 |

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**Table A-7 Estimated Daily Intake of Astaxanthin from Individual Proposed Food-Uses by the Total Population (All Ages) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption | | All-Users Consumption | |
|---|---|---|---|---|---|---|
| | | | Mean (mg) | 90th Percentile (mg) | Mean (mg) | 90th Percentile (mg) |
| Frozen Dairy Desserts and Mixes | | | | | | |
| Frozen Yogurt | 1.5 | 124 | < 0.1 | na | 0.3 | 0.7 |
| Ice Cream and Frozen Milk Desserts | 24.9 | 2,061 | 0.1 | 0.3 | 0.3 | 0.5 |
| Hard Candy | | | | | | |
| Hard Candy | 9.8 | 808 | < 0.1 | na | 0.4 | 0.8 |
| Milk Products | | | | | | |
| Dry Milks | 8.6 | 709 | < 0.1 | na | 0.2 | 0.2 |
| Flavored Milk and Milk Drinks | 15.0 | 1,238 | 0.1 | 0.2 | 0.4 | 0.8 |
| Milk-Based Meal Replacements, Ready-to-Drink | 1.3 | 104 | < 0.1 | na | 0.7 | 1.4 |
| Yogurt | 8.6 | 711 | < 0.1 | na | 0.2 | 0.3 |
| Processed Fruits and Fruit Juices | | | | | | |
| Fruit Drinks and Ades | 38.5 | 3,179 | 0.2 | 0.7 | 0.6 | 1.4 |
| Processed Vegetables and Vegetable Juices | | | | | | |
| Vegetable Juices | 4.0 | 328 | < 0.1 | na | 0.2 | 0.6 |
| Soft Candy | | | | | | |
| Chocolate Confections and Other Soft Candy | 31.8 | 2,628 | 0.1 | 0.4 | 0.3 | 0.7 |

na = not applicable

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## APPENDIX B

**Estimated Daily per Kilogram Body Weight Intake of Astaxanthin from Individual Proposed Food-Uses by Different Population Groups Within the United States**

**Table B-1 Estimated Daily Per Kilogram Body Weight Intake of Astaxanthin by Infants (Aged 0 to 2 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption (µg/kg) | | All-User Consumption (µg/kg) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Baked Goods and Baking Mixes | | | | | | |
| Cookies | 39.9 | 371 | 9 | 27 | 19 | 41 |
| Crackers, Savory | 30.4 | 283 | 6 | 20 | 16 | 30 |
| Crackers, Sweet | 17.3 | 161 | 4 | 15 | 16 | 32 |
| Yeast Breads and Rolls | 41.9 | 390 | 10 | 32 | 19 | 37 |
| Beverages and Beverage Bases | | | | | | |
| Bottled, Enhanced, and Carbonated Water | 0.6 | 6 | <1* | na | 4* | 10* |
| Carbonated Beverages | 22.3 | 207 | 4 | 12 | 18 | 45 |
| Energy, Sports, and Isotonic Drinks | 4.7 | 44 | 2 | na | 48 | 103 |
| Non-Milk Based Meal Replacements | 0.2 | 2 | <1* | na | 22* | 23* |
| Cereals and Cereal Products | | | | | | |
| Cereal and Energy Bars | 2.3 | 21 | 1* | na | 21* | 27* |
| Instant and Hot Cereals | 11.8 | 110 | 2 | 4 | 19 | 38 |
| Ready-to-Eat Cereals | 41.4 | 385 | 11 | 32 | 21 | 40 |
| Chewing Gum | | | | | | |
| Chewing Gum | 1.4 | 13 | <1* | na | 32* | 50* |
| Coffee and Tea | | | | | | |
| Coffee (Instant and Regular) | 1.2 | 11 | <1* | na | 7* | 19* |
| Tea (Instant, Regular, and Specialty) | 5.6 | 52 | 2 | na | 31 | 44 |
| Dairy Product Analogs | | | | | | |
| Imitation and Soy Milk | 0.5 | 5 | 1* | na | 51* | 133* |

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**Table B-1 Estimated Daily Per Kilogram Body Weight Intake of Astaxanthin by Infants (Aged 0 to 2 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption (µg/kg) | | All-User Consumption (µg/kg) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Frozen Dairy Desserts and Mixes | | | | | | |
| Frozen Yogurt | 1.0 | 9 | <1* | na | 13* | 20* |
| Ice Cream and Frozen Milk Desserts | 15.1 | 140 | 2 | 7 | 10 | 21 |
| Hard Candy | | | | | | |
| Hard Candy | 8.8 | 82 | 1 | na | 14 | 30 |
| Milk Products | | | | | | |
| Dry Milks | 5.9 | 55 | <1 | na | 1 | 4 |
| Flavored Milk and Milk Drinks | 9.7 | 90 | 4 | 10 | 28 | 82 |
| Milk-Based Meal Replacements, Ready-to-Drink | 0.1 | 1 | <1* | na | 4* | 4* |
| Yogurt | 13.0 | 121 | 2 | 11 | 12 | 22 |
| Processed Fruits and Fruit Juices | | | | | | |
| Fruit Drinks and Ades | 29.2 | 272 | 11 | 34 | 37 | 78 |
| Processed Vegetables and Vegetable Juices | | | | | | |
| Vegetable Juices | 6.1 | 57 | <1 | na | 7 | 14 |
| Soft Candy | | | | | | |
| Chocolate Confections and Other Soft Candy | 18.8 | 175 | 4 | 11 | 14 | 35 |

na = not applicable
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% or the sample size is less than 30 (see Section 2.3).

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**Table B-2 Estimated Daily Per Kilogram Body Weight Intake of Astaxanthin by Children (Aged 3 to 11 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption (µg/kg) | | All-User Consumption (µg/kg) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Baked Goods and Baking Mixes | | | | | | |
| Cookies | 49.8 | 641 | 7 | 19 | 13 | 27 |
| Crackers, Savory | 25.8 | 332 | 3 | 11 | 10 | 22 |
| Crackers, Sweet | 11.7 | 151 | 1 | 4 | 10 | 23 |
| Yeast Breads and Rolls | 83.5 | 1,075 | 15 | 33 | 17 | 34 |
| Beverages and Beverage Bases | | | | | | |
| Bottled, Enhanced, and Carbonated Water | 0.9 | 12 | <1* | na | 15* | 35* |
| Carbonated Beverages | 63.1 | 812 | 14 | 35 | 21 | 38 |
| Energy, Sports, and Isotonic Drinks | 7.5 | 97 | 1 | na | 15 | 34 |
| Non-Milk Based Meal Replacements | 0.4 | 5 | <1* | na | 19* | 26* |
| Cereals and Cereal Products | | | | | | |
| Cereal and Energy Bars | 4.1 | 53 | 1 | na | 12 | 21 |
| Instant and Hot Cereals | 7.4 | 95 | 1 | na | 12 | 23 |
| Ready-to-Eat Cereals | 66.0 | 849 | 13 | 33 | 20 | 39 |
| Chewing Gum | | | | | | |
| Chewing Gum | 6.4 | 82 | 1 | na | 17 | 34 |
| Coffee and Tea | | | | | | |
| Coffee (Instant and Regular) | 2.8 | 36 | <1* | na | 6 | 10 |
| Tea (Instant, Regular, and Specialty) | 13.1 | 168 | 4 | 8 | 23 | 89 |
| Dairy Product Analogs | | | | | | |
| Imitation and Soy Milk | 0.5 | 7 | <1* | na | 27* | 85* |

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**Table B-2 Estimated Daily Per Kilogram Body Weight Intake of Astaxanthin by Children (Aged 3 to 11 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption (µg/kg) | | All-User Consumption (µg/kg) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Frozen Dairy Desserts and Mixes | | | | | | |
| Frozen Yogurt | 1.3 | 17 | <1* | na | 9* | 15* |
| Ice Cream and Frozen Milk Desserts | 33.8 | 435 | 3 | 10 | 8 | 17 |
| Hard Candy | | | | | | |
| Hard Candy | 18.0 | 232 | 2 | 8 | 13 | 31 |
| Milk Products | | | | | | |
| Dry Milks | 11.0 | 142 | <1 | <1 | 3 | 7 |
| Flavored Milk and Milk Drinks | 34.0 | 438 | 6 | 17 | 15 | 29 |
| Milk-Based Meal Replacements, Ready-to-Drink | 0.3 | 4 | <1* | na | 8* | 19* |
| Yogurt | 12.8 | 165 | 1 | 4 | 8 | 12 |
| Processed Fruits and Fruit Juices | | | | | | |
| Fruit Drinks and Ades | 61.6 | 793 | 11 | 31 | 21 | 42 |
| Processed Vegetables and Vegetable Juices | | | | | | |
| Vegetable Juices | 2.9 | 37 | <1* | na | 5 | 17 |
| Soft Candy | | | | | | |
| Chocolate Confections and Other Soft Candy | 40.6 | 522 | 4 | 15 | 10 | 22 |

na = not applicable
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% or the sample size is less than 30 (see Section 2.3).



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**Table B-3 Estimated Daily Per Kilogram Body Weight Intake of Astaxanthin by Female Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption (µg/kg) | | All-User Consumption (µg/kg) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Baked Goods and Baking Mixes | | | | | | |
| Cookies | 35.8 | 355 | 2 | 7 | 6 | 14 |
| Crackers, Savory | 16.2 | 161 | 1 | 4 | 5 | 10 |
| Crackers, Sweet | 4.9 | 49 | <1 | na | 5 | 14 |
| Yeast Breads and Rolls | 78.6 | 780 | 7 | 16 | 9 | 18 |
| Beverages and Beverage Bases | | | | | | |
| Bottled, Enhanced, and Carbonated Water | 1.9 | 19 | <1* | na | 5* | 7* |
| Carbonated Beverages | 75.6 | 750 | 14 | 33 | 17 | 36 |
| Energy, Sports, and Isotonic Drinks | 6.5 | 64 | 1 | na | 8 | 14 |
| Non-Milk Based Meal Replacements | 0.3 | 3 | <1* | na | 4* | 7* |
| Cereals and Cereal Products | | | | | | |
| Cereal and Energy Bars | 6.5 | 64 | 1 | na | 6 | 11 |
| Instant and Hot Cereals | 2.7 | 27 | <1* | na | 5* | 9* |
| Ready-to-Eat Cereals | 41.7 | 414 | 5 | 14 | 12 | 23 |
| Chewing Gum | | | | | | |
| Chewing Gum | 9.8 | 97 | 1 | na | 13 | 35 |
| Coffee and Tea | | | | | | |
| Coffee (Instant and Regular) | 10.8 | 107 | 1 | 4 | 8 | 14 |
| Tea (Instant, Regular, and Specialty) | 21.4 | 212 | 2 | 10 | 11 | 21 |
| Dairy Product Analogs | | | | | | |
| Imitation and Soy Milk | 0.7 | 7 | <1* | na | 11* | 14* |



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**Table B-3 Estimated Daily Per Kilogram Body Weight Intake of Astaxanthin by Female Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption (µg/kg) | | All-User Consumption (µg/kg) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Frozen Dairy Desserts and Mixes | | | | | | |
| Frozen Yogurt | 1.1 | 11 | <1* | na | 4* | 8* |
| Ice Cream and Frozen Milk Desserts | 25.5 | 253 | 1 | 4 | 5 | 10 |
| Hard Candy | | | | | | |
| Hard Candy | 12.6 | 125 | <1 | 1 | 5 | 12 |
| Milk Products | | | | | | |
| Dry Milks | 9.6 | 95 | <1 | <1 | 1 | 2 |
| Flavored Milk and Milk Drinks | 18.6 | 185 | 1 | 4 | 7 | 16 |
| Milk-Based Meal Replacements, Ready-to-Drink | 0.8 | 8 | <1* | na | 8* | 12* |
| Yogurt | 7.0 | 69 | <1 | na | 4 | 8 |
| Processed Fruits and Fruit Juices | | | | | | |
| Fruit Drinks and Ades | 49.2 | 488 | 4 | 12 | 10 | 20 |
| Processed Vegetables and Vegetable Juices | | | | | | |
| Vegetable Juices | 1.6 | 16 | <1* | na | 5* | 12* |
| Soft Candy | | | | | | |
| Chocolate Confections and Other Soft Candy | 41.2 | 409 | 2 | 9 | 6 | 13 |

na = not applicable
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% or the sample size is less than 30 (see Section 2.3).



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**Table B-4 Estimated Daily Per Kilogram Body Weight Intake of Astaxanthin by Male Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption (µg/kg) | | All-User Consumption (µg/kg) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Baked Goods and Baking Mixes | | | | | | |
| Cookies | 34.3 | 343 | 2 | 8 | 7 | 14 |
| Crackers, Savory | 12.1 | 121 | 1 | 1 | 7 | 15 |
| Crackers, Sweet | 3.9 | 39 | <1 | na | 4 | 9 |
| Yeast Breads and Rolls | 83.0 | 829 | 9 | 18 | 10 | 20 |
| Beverages and Beverage Bases | | | | | | |
| Bottled, Enhanced, and Carbonated Water | 2.2 | 22 | <1* | na | 7* | 10* |
| Carbonated Beverages | 81.0 | 809 | 17 | 36 | 20 | 38 |
| Energy, Sports, and Isotonic Drinks | 11.9 | 119 | 2 | 9 | 14 | 28 |
| Non-Milk Based Meal Replacements | 0.3 | 3 | <1* | na | 6* | 6* |
| Cereals and Cereal Products | | | | | | |
| Cereal and Energy Bars | 4.2 | 42 | <1 | na | 6 | 13 |
| Instant and Hot Cereals | 2.0 | 20 | <1* | na | 4* | 8* |
| Ready-to-Eat Cereals | 43.6 | 436 | 6 | 17 | 13 | 24 |
| Chewing Gum | | | | | | |
| Chewing Gum | 8.5 | 85 | 1 | na | 9 | 15 |
| Coffee and Tea | | | | | | |
| Coffee (Instant and Regular) | 6.8 | 68 | 1 | na | 12 | 49 |
| Tea (Instant, Regular, and Specialty) | 18.4 | 184 | 3 | 10 | 10 | 16 |
| Dairy Product Analogs | | | | | | |
| Imitation and Soy Milk | 0.3 | 3 | <1* | na | 6* | 9* |

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**Table B-4 Estimated Daily Per Kilogram Body Weight Intake of Astaxanthin by Male Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption (µg/kg) | | All-User Consumption (µg/kg) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Frozen Dairy Desserts and Mixes | | | | | | |
| Frozen Yogurt | 0.4 | 4 | <1* | na | 12* | 35* |
| Ice Cream and Frozen Milk Desserts | 23.3 | 233 | 1 | 5 | 5 | 10 |
| Hard Candy | | | | | | |
| Hard Candy | 9.4 | 94 | 1 | 1 | 5 | 11 |
| Milk Products | | | | | | |
| Dry Milks | 7.0 | 70 | <1 | na | 1 | 4 |
| Flavored Milk and Milk Drinks | 19.3 | 193 | 1 | 5 | 8 | 15 |
| Milk-Based Meal Replacements, Ready-to-Drink | 0.7 | 7 | <1* | na | 10* | 10* |
| Yogurt | 3.2 | 32 | <1 | na | 4 | 7 |
| Processed Fruits and Fruit Juices | | | | | | |
| Fruit Drinks and Ades | 48.1 | 481 | 5 | 18 | 13 | 26 |
| Processed Vegetables and Vegetable Juices | | | | | | |
| Vegetable Juices | 1.5 | 15 | <1* | na | 10* | 15* |
| Soft Candy | | | | | | |
| Chocolate Confections and Other Soft Candy | 32.9 | 329 | 2 | 7 | 7 | 18 |

na = not applicable
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% or the sample size is less than 30 (see Section 2.3).

660000

**Table B-5 Estimated Daily Per Kilogram Body Weight Intake of Astaxanthin by Female Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption (µg/kg) | | All-User Consumption (µg/kg) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Baked Goods and Baking Mixes | | | | | | |
| Cookies | 34.6 | 737 | 2 | 6 | 5 | 12 |
| Crackers, Savory | 24.1 | 514 | 1 | 3 | 3 | 6 |
| Crackers, Sweet | 7.1 | 151 | <1 | na | 3 | 9 |
| Yeast Breads and Rolls | 82.6 | 1,758 | 6 | 12 | 7 | 13 |
| Beverages and Beverage Bases | | | | | | |
| Bottled, Enhanced, and Carbonated Water | 2.9 | 62 | <1 | na | 10 | 23 |
| Carbonated Beverages | 63.8 | 1,358 | 10 | 27 | 15 | 32 |
| Energy, Sports, and Isotonic Drinks | 1.6 | 35 | <1 | na | 13 | 49 |
| Non-Milk Based Meal Replacements | 1.2 | 26 | <1* | na | 5* | 9* |
| Cereals and Cereal Products | | | | | | |
| Cereal and Energy Bars | 5.9 | 126 | <1 | na | 5 | 9 |
| Instant and Hot Cereals | 13.4 | 285 | <1 | <1 | 4 | 8 |
| Ready-to-Eat Cereals | 32.7 | 697 | 2 | 8 | 7 | 12 |
| Chewing Gum | | | | | | |
| Chewing Gum | 3.7 | 79 | <1 | na | 8 | 13 |
| Coffee and Tea | | | | | | |
| Coffee (Instant and Regular) | 58.9 | 1,253 | 8 | 22 | 14 | 27 |
| Tea (Instant, Regular, and Specialty) | 35.8 | 762 | 5 | 16 | 13 | 27 |
| Dairy Product Analogs | | | | | | |
| Imitation and Soy Milk | 2.7 | 58 | <1 | na | 7 | 19 |

000100

**Table B-5 Estimated Daily Per Kilogram Body Weight Intake of Astaxanthin by Female Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption (µg/kg) | | All-User Consumption (µg/kg) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Frozen Dairy Desserts and Mixes | | | | | | |
| Frozen Yogurt | 2.2 | 47 | <1 | na | 5 | 13 |
| Ice Cream and Frozen Milk Desserts | 25.2 | 537 | 1 | 3 | 4 | 7 |
| Hard Candy | | | | | | |
| Hard Candy | 7.5 | 160 | 1 | na | 7 | 14 |
| Milk Products | | | | | | |
| Dry Milks | 8.8 | 187 | <1* | na | 5* | 5* |
| Flavored Milk and Milk Drinks | 9.2 | 196 | 1 | na | 7 | 13 |
| Milk-Based Meal Replacements, Ready-to-Drink | 2.3 | 49 | <1 | na | 7 | 14 |
| Yogurt | 10.1 | 216 | <1 | <1 | 3 | 5 |
| Processed Fruits and Fruit Juices | | | | | | |
| Fruit Drinks and Ades | 28.7 | 612 | 2 | 7 | 8 | 17 |
| Processed Vegetables and Vegetable Juices | | | | | | |
| Vegetable Juices | 5.4 | 115 | <1 | na | 3 | 6 |
| Soft Candy | | | | | | |
| Chocolate Confections and Other Soft Candy | 32.5 | 691 | 2 | 5 | 4 | 8 |

na = not applicable
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% or the sample size is less than 30 (see Section 2.3).

000101

**Table B-6 Estimated Daily Per Kilogram Body Weight Intake of Astaxanthin by Male Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption (µg/kg) | | All-User Consumption (µg/kg) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Baked Goods and Baking Mixes | | | | | | |
| Cookies | 33.2 | 640 | 2 | 6 | 6 | 12 |
| Crackers, Savory | 18.9 | 365 | 1 | 2 | 4 | 7 |
| Crackers, Sweet | 5.4 | 104 | <1 | na | 3 | 6 |
| Yeast Breads and Rolls | 85.9 | 1,657 | 7 | 15 | 8 | 16 |
| Beverages and Beverage Bases | | | | | | |
| Bottled, Enhanced, and Carbonated Water | 1.9 | 36 | <1 | na | 7 | 9 |
| Carbonated Beverages | 69.2 | 1,335 | 13 | 30 | 17 | 35 |
| Energy, Sports, and Isotonic Drinks | 6.1 | 117 | 1 | na | 10 | 19 |
| Non-Milk Based Meal Replacements | 1.9 | 37 | <1 | na | 9 | 17 |
| Cereals and Cereal Products | | | | | | |
| Cereal and Energy Bars | 5.1 | 98 | <1 | na | 4 | 8 |
| Instant and Hot Cereals | 10.3 | 199 | <1 | na | 5 | 9 |
| Ready-to-Eat Cereals | 30.0 | 578 | 3 | 9 | 8 | 16 |
| Chewing Gum | | | | | | |
| Chewing Gum | 3.2 | 62 | <1 | na | 5 | 11 |
| Coffee and Tea | | | | | | |
| Coffee (Instant and Regular) | 62.9 | 1,214 | 8 | 22 | 14 | 27 |
| Tea (Instant, Regular, and Specialty) | 29.8 | 574 | 4 | 13 | 14 | 30 |
| Dairy Product Analogs | | | | | | |
| Imitation and Soy Milk | 2.0 | 39 | <1 | na | 5 | 10 |
| Frozen Dairy Desserts and Mixes | | | | | | |
| Frozen Yogurt | 1.9 | 36 | <1 | na | 4 | 7 |
| Ice Cream and Frozen Milk Desserts | 24.0 | 463 | 1 | 4 | 4 | 7 |

**Table B-6 Estimated Daily Per Kilogram Body Weight Intake of Astaxanthin by Male Adults (Aged 20 Years and Over) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption (µg/kg) | | All-User Consumption (µg/kg) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Hard Candy | | | | | | |
| Hard Candy | 6.0 | 115 | <1 | na | 5 | 12 |
| Milk Products | | | | | | |
| Dry Milks | 8.3 | 160 | <1* | na | 1* | 2* |
| Flavored Milk and Milk Drinks | 7.1 | 136 | <1 | na | 5 | 11 |
| Milk-Based Meal Replacements, Ready-to-Drink | 1.8 | 35 | <1 | na | 12 | 31 |
| Yogurt | 5.6 | 108 | <1 | na | 2 | 4 |
| Processed Fruits and Fruit Juices | | | | | | |
| Fruit Drinks and Ades | 27.6 | 533 | 2 | 7 | 9 | 20 |
| Processed Vegetables and Vegetable Juices | | | | | | |
| Vegetable Juices | 4.6 | 88 | <1 | na | 3 | 7 |
| Soft Candy | | | | | | |
| Chocolate Confections and Other Soft Candy | 26.0 | 502 | 1 | 5 | 4 | 9 |

na = not applicable
*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% or the sample size is less than 30 (see Section 2.3).

000103

**Table B-7 Estimated Daily Per Kilogram Body Weight Intake of Astaxanthin from Individual Proposed Food-Uses by the Total Population (All Ages) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption (µg/kg) | | All-User Consumption (µg/kg) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Baked Goods and Baking Mixes | | | | | | |
| Cookies | 37.3 | 3,087 | 3 | 9 | 8 | 17 |
| Crackers, Savory | 21.5 | 1,776 | 1 | 4 | 6 | 13 |
| Crackers, Sweet | 7.9 | 655 | <1 | na | 7 | 16 |
| Yeast Breads and Rolls | 78.5 | 6,489 | 8 | 17 | 9 | 18 |
| Beverages and Beverage Bases | | | | | | |
| Bottled, Enhanced, and Carbonated Water | 1.9 | 157 | <1 | na | 9 | 19 |
| Carbonated Beverages | 63.8 | 5,271 | 12 | 30 | 17 | 36 |
| Energy, Sports, and Isotonic Drinks | 5.8 | 476 | 1 | na | 14 | 29 |
| Non-Milk Based Meal Replacements | 0.9 | 76 | <1 | na | 7 | 17 |
| Cereals and Cereal Products | | | | | | |
| Cereal and Energy Bars | 4.8 | 400 | <1 | na | 6 | 14 |
| Instant and Hot Cereals | 8.9 | 736 | <1 | na | 6 | 11 |
| Ready-to-Eat Cereals | 40.6 | 3,359 | 4 | 14 | 12 | 25 |
| Chewing Gum | | | | | | |
| Chewing Gum | 5.1 | 418 | <1 | na | 10 | 20 |
| Coffee and Tea | | | | | | |
| Coffee (Instant and Regular) | 32.5 | 2,689 | 6 | 18 | 14 | 27 |
| Tea (Instant, Regular, and Specialty) | 23.6 | 1,952 | 4 | 13 | 14 | 29 |
| Dairy Product Analogs | | | | | | |
| Imitation and Soy Milk | 1.4 | 119 | <1 | na | 8 | 19 |

**Table B-7 Estimated Daily Per Kilogram Body Weight Intake of Astaxanthin from Individual Proposed Food-Uses by the Total Population (All Ages) Within the United States (2003-2004 NHANES Data)**

| Food-Use Category | % Users | Actual # of Total Users | All-Person Consumption (µg/kg) | | All-User Consumption (µg/kg) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90th Percentile | Mean | 90th Percentile |
| Frozen Dairy Desserts and Mixes | | | | | | |
| Frozen Yogurt | 1.5 | 124 | <1 | na | 5 | 10 |
| Ice Cream and Frozen Milk Desserts | 24.9 | 2,061 | 1 | 4 | 5 | 10 |
| Hard Candy | | | | | | |
| Hard Candy | 9.8 | 808 | 1 | na | 8 | 19 |
| Milk Products | | | | | | |
| Dry Milks | 8.6 | 709 | <1 | na | 3 | 4 |
| Flavored Milk and Milk Drinks | 15.0 | 1,238 | 1 | 4 | 11 | 21 |
| Milk-Based Meal Replacements, Ready-to-Drink | 1.3 | 104 | <1 | na | 9 | 19 |
| Yogurt | 8.6 | 711 | <1 | na | 5 | 10 |
| Processed Fruits and Fruit Juices | | | | | | |
| Fruit Drinks and Ades | 38.5 | 3,179 | 4 | 12 | 13 | 29 |
| Processed Vegetables and Vegetable Juices | | | | | | |
| Vegetable Juices | 4.0 | 328 | <1 | na | 4 | 7 |
| Soft Candy | | | | | | |
| Chocolate Confections and Other Soft Candy | 31.8 | 2,628 | 2 | 6 | 6 | 12 |

na = not applicable

000105

## APPENDIX C

**Representative NHANES 2003-2004 Food Codes for All Proposed Food-Uses of Astaxanthin in the United States**

# Representative NHANES 2003-2004 Food Codes for All Proposed Food-Uses of Astaxanthin in the United States

## Baked Goods and Baking Mixes

**Cookies**
[Astaxanthin] = 0.0017%

| | |
|---|---|
| 53200100 | Cookie, batter or dough, raw, not chocolate |
| 53201000 | Cookie, NS as to type |
| 53202000 | Cookie, almond |
| 53203000 | Cookie, applesauce |
| 53203050 | Cookie, fruit, baby |
| 53203100 | Cookie, baby |
| 53203500 | Cookie, biscotti (Italian sugar cookie) |
| 53205250 | Cookie, butterscotch, brownie |
| 53205500 | Cookie, butterscotch chip |
| 53205600 | Cookie, caramel coated, with nuts |
| 53205760 | Cookie, carob and honey brownie |
| 53206000 | Cookie, chocolate chip |
| 53206010 | Cookie, chocolate chip, with raisins |
| 53206020 | Cookie, chocolate chip, made from home recipe or purchased at a bakery |
| 53206030 | Cookie, chocolate chip, reduced fat |
| 53206050 | Cookie, rich, chocolate chip, with chocolate filling |
| 53206100 | Cookie, chocolate chip sandwich |
| 53206500 | Cookie, chocolate, made with rice cereal (no-bake) |
| 53206550 | Cookie, chocolate, made with oatmeal and coconut (no-bake) |
| 53207000 | Cookie, chocolate fudge, with/without nuts |
| 53208000 | Cookie, chocolate-covered marshmallow |
| 53208200 | Cookie, marshmallow pie, chocolate covered |
| 53209000 | Cookie, chocolate, chocolate sandwich or chocolate-coated or striped |
| 53209010 | Cookie, chocolate-covered, sugar wafer, creme- or caramel-filled |
| 53209020 | Cookie, chocolate sandwich, reduced fat |
| 53209050 | Cookie, chocolate-covered, chocolate sandwich |
| 53209100 | Cookie, chocolate, sandwich, with extra filling |
| 53209500 | Cookie, chocolate and vanilla sandwich |
| 53210000 | Cookie, chocolate wafer |
| 53210900 | Cookie, graham cracker sandwich with chocolate and marshmallow filling |
| 53210910 | Cookie, graham cracker with marshmallow |
| 53215500 | Cookie, coconut |
| 53216000 | Cookie, coconut and nut |
| 53220000 | Cookie, fruit-filled bar |
| 53220020 | Cookie, date bar |
| 53220030 | Cookie, fig bar |
| 53222010 | Cookie, fortune |
| 53222020 | Cookie, cone shell, ice cream type, wafer or cake |
| 53222100 | Cookie, cone shell, ice cream type, brown sugar |
| 53223000 | Cookie, gingersnaps |
| 53223100 | Cookie, granola |
| 53224000 | Cookie, ladyfinger |
| 53224250 | Cookie, lemon bar |
| 53225000 | Cookie, macaroon, coconut-meringue type, no flour |
| 53226000 | Cookie, marshmallow, with coconut |
| 53226500 | Cookie, marshmallow, with rice cereal (no-bake) |
| 53226550 | Cookie, marshmallow, with rice cereal and chocolate chips |

000107

| | |
|---|---|
| 53226600 | Cookie, marshmallow and peanut butter, with oat cereal (no-bake) |
| 53227000 | Cookie, marshmallow pies, non-chocolate coating |
| 53228000 | Cookie, meringue |
| 53230000 | Cookie, molasses |
| 53231000 | Cookie, Lebkuchen |
| 53231400 | Cookie, multigrain, high fiber |
| 53233000 | Cookie, oatmeal |
| 53233010 | Cookie, oatmeal, with raisins |
| 53233020 | Cookie, oatmeal, with fruit filling |
| 53233030 | Cookie, oatmeal, fat free, with raisins |
| 53233040 | Cookie, oatmeal, reduced fat, with raisins |
| 53233050 | Cookie, oatmeal sandwich, with creme filling |
| 53233060 | Cookie, oatmeal, with chocolate chips |
| 53233080 | Cookie, oatmeal sandwich, with peanut butter and jelly filling |
| 53233100 | Cookie, oatmeal, with chocolate and peanut butter (no-bake) |
| 53234000 | Cookie, peanut butter |
| 53234010 | Cookie, peanut butter, with oatmeal |
| 53234100 | Cookie, peanut butter, with chocolate |
| 53234250 | Cookie, peanut butter with rice cereal (no-bake) |
| 53235000 | Cookie, peanut |
| 53235500 | Cookie, with peanut butter filling, chocolate-coated |
| 53235600 | Cookie, Pfeffernusse |
| 53236000 | Cookie, pizzelle (Italian style wafer) |
| 53236100 | Cookie, pumpkin |
| 53237000 | Cookie, raisin |
| 53237010 | Cookie, raisin sandwich, cream-filled |
| 53237500 | Cookie, rum ball (no-bake) |
| 53238000 | Cookie, sandwich-type, not chocolate or vanilla |
| 53239000 | Cookie, shortbread |
| 53239010 | Cookie, shortbread, reduced fat |
| 53239050 | Cookie, shortbread, with chocolate filling |
| 53241500 | Cookie, butter or sugar cookie |
| 53241600 | Cookie, butter or sugar cookie, with fruit and/or nuts |
| 53242000 | Cookie, sugar wafer |
| 53242250 | Cookie, teething, baby food |
| 53242500 | Cookie, toffee bar |
| 53243000 | Cookie, vanilla sandwich |
| 53243100 | Cookie, rich, all chocolate, with chocolate filling or chocolate chips |
| 53244010 | Cookie, butter or sugar, with chocolate icing or filling |
| 53244020 | Cookie, butter or sugar, iced, with icing other than chocolate |
| 53245000 | Cookie, vanilla waffle creme |
| 53246000 | Cookie, tea, Japanese |
| 53247000 | Cookie, vanilla wafer |
| 53247050 | Cookie, vanilla wafer, reduced fat |
| 53247500 | Cookie, vanilla with caramel, coconut, and chocolate coating |
| 53248000 | Cookie, whole wheat, dried fruit, nut |
| 53251100 | Cookie, rugelach |
| 53260030 | Cookie, dietetic, chocolate chip |
| 53260150 | Cookie, lemon wafer, lowfat |
| 53260200 | Cookie, dietetic, oatmeal with raisins |
| 53260300 | Cookie, dietetic, sandwich type |
| 53260400 | Cookie, dietetic, sugar or plain |
| 53270100 | Cookies, Puerto Rican (Mantecaditos polvorones) |

000108

**Mixtures containing cookies**
(Adjusted for cookie content of 90.0 to 98.0%)
[Astaxanthin] = 0.0015 to 0.00167%

| | |
|---|---|
| 53207050 | Cookie, chocolate, with chocolate filling or coating, fat free |
| 53220040 | Cookie, fig bar, fat free |
| 53220010 | Cookie, fruit-filled bar, fat free |
| 53243050 | Cookie, vanilla sandwich, reduced fat |
| 53205750 | Cookie, carob |
| 53233500 | Cookie, oat bran |

**Mixtures containing cookies**
(Adjusted for cookie content of 9.2 to 62.0%)
[Astaxanthin] = 0.00016 to 0.0010%

| | |
|---|---|
| 13161520 | Milk dessert sandwich bar, frozen, with low-calorie sweetener, made from lowfat milk |
| 53371100 | Pie, chiffon, with liqueur |
| 28150650 | Fish, breaded, or fish sticks, with pasta, vegetable and dessert (frozen meal) |
| 28140740 | Chicken patty, or nuggets, boneless, breaded, with pasta and tomato sauce, fruit, dessert (frozen meal) |
| 13241000 | Pudding, with fruit and vanilla wafers |
| 53373000 | Pie, black bottom |

**Cookies (brownie)**
[Astaxanthin] = 0.0013%

| | |
|---|---|
| 53204000 | Cookie, brownie, NS as to icing |
| 53204010 | Cookie, brownie, without icing |
| 53204100 | Cookie, brownie, with icing |
| 53204500 | Cookie, brownie, with cream cheese filling, without icing |
| 53204600 | Cookie, brownie, with peanut butter fudge icing |
| 53204800 | Cookie, brownie, diet, NS as to icing |
| 53204830 | Cookie, brownie, lowfat, with icing |
| 53204840 | Cookie, brownie, lowfat, without icing |
| 53204850 | Cookie, brownie, fat free, cholesterol free, with icing |
| 53204860 | Cookie, brownie, fat free, without icing |

**Crackers, Savory**
[Astaxanthin] = 0.0033%

| | |
|---|---|
| 51187000 | Melba toast |

[Astaxanthin] = 0.0017%

| | |
|---|---|
| 54001000 | Crackers, NS as to sweet or nonsweet |
| 54202010 | Crackers, saltine, low sodium |
| 54202050 | Crackers, saltine, fat free, low sodium |
| 54203010 | Crackers, toast thins (rye, wheat, white flour), low sodium |
| 54204010 | Cracker, 100% whole wheat, low sodium |
| 54205010 | Cracker, snack, low sodium |
| 54205030 | Cracker, cheese, low sodium |
| 54210010 | Cracker, multigrain, salt free |
| 54222000 | Crispbread, rye, low sodium |
| 54301000 | Cracker, snack |
| 54304000 | Cracker, cheese |

| | |
|---|---|
| 54304500 | Cracker, high fiber, no added fat |
| 54307000 | Crackers, matzo |
| 54308000 | Crackers, milk |
| 54309000 | Crackers, oat |
| 54313000 | Crackers, oyster |
| 54319000 | Crackers, rice |
| 54322000 | Crispbread, rye, no added fat |
| 54325000 | Crackers, saltine |
| 54325050 | Crackers, saltine, whole wheat |
| 54327950 | Crackers, cylindrical, peanut-butter filled |
| 54328000 | Crackers, sandwich-type, NFS |
| 54328100 | Cracker, sandwich-type, peanut butter filled |
| 54328200 | Cracker, sandwich-type, cheese-filled |
| 54334000 | Crackers, toast thins (rye, pumpernickel, white flour) |
| 54336000 | Crackers, water biscuits |
| 54337000 | Cracker, 100% whole wheat |
| 54338000 | Crackers, wheat |
| 54338100 | Crackers, wheat, reduced fat |
| 54339000 | Crackers, corn |
| 54350000 | Crackers, baby food |

**Mixtures containing crackers, savoury**
(Adjusted for cracker content of 92.5 to 98.5%)
[Astaxanthin] = 0.0016 to 0.0017%

| | |
|---|---|
| 54337100 | Crackers, whole wheat and bran |
| 54201010 | Crackers, matzo, low sodium |

**Mixtures containing crackers, savoury**
(Adjusted for cracker content of 0.72 to 19.4%)
[Astaxanthin] = 0.00001 to 0.0003%

| | |
|---|---|
| 54420010 | Multigrain mixture, pretzels, cereal and/or crackers, nuts |
| 52308010 | Matzo, fritters |
| 27220010 | Meat loaf made with ham (not luncheon meat) |
| 27148010 | Stuffed chicken, drumstick or breast, Puerto Rican style (Muslo de pollo o pechuga rellena) |
| 58404500 | Matzo ball soup |
| 75418220 | Creamed christophine, Puerto Rican style (Chayote a la crema) |
| 58157110 | Spicey rice pudding, Puerto Rican style (arroz con dulce, arroz con especie) |
| 24302010 | Duck, pressed, Chinese |
| 58146200 | Pasta, meat-filled, with gravy, canned |

**Crackers, Sweet**
[Astaxanthin] = 0.0017%

| | |
|---|---|
| 54101010 | Cracker, animal |
| 54102010 | Crackers, graham |
| 54102020 | Crackers, graham, chocolate covered |
| 54102050 | Crackers, oatmeal |
| 54102060 | Crackers, Cuban |
| 54102070 | Crackers, Cuca |
| 54102100 | Crackers, graham, lowfat |
| 54102110 | Crackers, graham, fat free |

000110

**Mixtures containing crackers, sweet**
(Adjusted for cracker content of 70.0 to 80.0%)
[Astaxanthin] = 0.0012 to 0.0014%

| | |
|---|---|
| 54102080 | Crackers, graham, with raisins |
| 54102200 | Crackers, graham, sandwich-type, with filling |

**Mixtures containing crackers, sweet**
(Adjusted for cracker content of 12.3 to 25.2%)
[Astaxanthin] = 0.0002 to 0.0004%

| | |
|---|---|
| 53104520 | Cheesecake, diet |
| 53111500 | Cake, graham cracker, without icing |
| 53104570 | Cheesecake, diet, with fruit |
| 53382000 | Pie, chocolate-marshmallow |
| 53366000 | Pie, yogurt, frozen |

**Mixtures containing crackers, sweet**
(Adjusted for cracker content of 1.4 to 10.3%)
[Astaxanthin] = 0.000024 to 0.00018%

| | |
|---|---|
| 53104500 | Cheesecake |
| 53390100 | Pie, tofu with fruit |
| 53104550 | Cheesecake with fruit |
| 53211000 | Cookie bar, with chocolate, nuts, and graham crackers |
| 28133340 | Veal parmigiana with vegetable, fettuccine alfredo, dessert (frozen meal) |
| 28140250 | Chicken, boneless, with gravy, dressing, rice, vegetable, dessert (frozen meal, large meat portion) |

**Yeast Breads and Rolls**
[Astaxanthin] = 0.001%

| | |
|---|---|
| 51000100 | Bread, NS as to major flour |
| 51000110 | Toast, NS as to major flour |
| 51000200 | Roll, NS as to major flour |
| 51000230 | Roll, NS as to major flour, toasted |
| 51000300 | Roll, hard, NS as to major flour |
| 51000400 | Roll, bran, NS as to type of bran |
| 51000410 | Roll, bran, NS as to type of bran, toasted |
| 51101000 | Bread, white |
| 51101010 | Bread, white, toasted |
| 51102010 | Bread, white with whole wheat swirl |
| 51102020 | Bread, white with whole wheat swirl, toasted |
| 51105010 | Bread, Cuban |
| 51105040 | Bread, Cuban, toasted |
| 51106010 | Bread, Native, Puerto Rican style (Pan Criollo) |
| 51106020 | Bread, Native, Puerto Rican style, toasted (Pan Criollo) |
| 51106100 | Bread, Native water, Puerto Rican style (Pan de agua) |
| 51106200 | Bread, lard, Puerto Rican style (Pan de manteca) |
| 51106210 | Bread, lard, Puerto Rican style, toasted (Pan de manteca) |
| 51106300 | Bread, caressed, Puerto Rican style (Pan sobao) |
| 51106310 | Bread, caressed, Puerto Rican style, toasted (Pan sobao) |
| 51107010 | Bread, French or Vienna |
| 51107040 | Bread, French or Vienna, toasted |
| 51108010 | Focaccia, Italian flatbread, plain |
| 51108100 | Naan, Indian flatbread |

| | |
|---|---|
| 51109010 | Bread, Italian, Grecian, Armenian |
| 51109040 | Bread, Italian, Grecian, Armenian, toasted |
| 51109100 | Bread, pita |
| 51109110 | Bread, pita, toasted |
| 51109150 | Bread, pita with fruit |
| 51109200 | Bread, pita with fruit, toasted |
| 51110010 | Bread, batter |
| 51111010 | Bread, cheese |
| 51111040 | Bread, cheese, toasted |
| 51113010 | Bread, cinnamon |
| 51113100 | Bread, cinnamon, toasted |
| 51115010 | Bread, cornmeal and molasses |
| 51115020 | Bread, cornmeal and molasses, toasted |
| 51119010 | Bread, egg, Challah |
| 51119040 | Bread, egg, Challah, toasted |
| 51119100 | Bread, lowfat, 98% fat free |
| 51119110 | Bread, lowfat, 98% fat free, toasted |
| 51121010 | Bread, garlic |
| 51121040 | Bread, garlic, toasted |
| 51121110 | Bread, onion |
| 51122000 | Bread, reduced calorie and/or high fiber, white or NFS |
| 51122010 | Bread, reduced calorie and/or high fiber, white or NFS, toasted |
| 51122050 | Bread, reduced calorie and/or high fiber, Italian |
| 51122060 | Bread, reduced calorie and/or high fiber, Italian, toasted |
| 51122100 | Bread, reduced calorie and/or high fiber, white or NFS, with fruit and/or nuts |
| 51122110 | Bread, reduced calorie and/or high fiber, white or NFS, with fruit and/or nuts, toasted |
| 51122300 | Bread, white, special formula, added fiber |
| 51122310 | Bread, white, special formula, added fiber, toasted |
| 51123010 | Bread, high protein |
| 51123020 | Bread, high protein, toasted |
| 51126010 | Bread, milk and honey |
| 51126020 | Bread, milk and honey, toasted |
| 51127010 | Bread, potato |
| 51127020 | Bread, potato, toasted |
| 51129010 | Bread, raisin |
| 51129020 | Bread, raisin, toasted |
| 51130510 | Bread, white, low sodium or no salt |
| 51130520 | Bread, white, low sodium or no salt, toasted |
| 51133010 | Bread, sour dough |
| 51133020 | Bread, sour dough, toasted |
| 51134000 | Bread, sweetpotato |
| 51135000 | Bread, vegetable |
| 51135010 | Bread, vegetable, toasted |
| 51140100 | Bread, dough, fried |
| 51150000 | Roll, white, soft |
| 51150100 | Roll, white, soft, toasted |
| 51152000 | Roll, white, soft, reduced calorie and/or high fiber |
| 51152100 | Roll, white, soft, reduced calorie and/or high fiber, toasted |
| 51153000 | Roll, white, hard |
| 51153010 | Roll, white, hard, toasted |
| 51154510 | Roll, diet |
| 51154550 | Roll, egg bread |
| 51154560 | Roll, egg bread, toasted |
| 51154600 | Roll, cheese |
| 51155000 | Roll, French or Vienna |

| | |
|---|---|
| 51155010 | Roll, French or Vienna, toasted |
| 51156500 | Roll, garlic |
| 51157000 | Roll, hoagie, submarine |
| 51157010 | Roll, hoagie, submarine, toasted |
| 51158100 | Roll, Mexican, bolillo |
| 51159000 | Roll, sour dough |
| 51201010 | Bread, whole wheat, 100% |
| 51201020 | Bread, whole wheat, 100%, toasted |
| 51201110 | Bread, whole wheat, 100%, with raisins |
| 51201120 | Bread, whole wheat, 100%, with raisins, toasted |
| 51201150 | Bread, pita, whole wheat, 100% |
| 51201160 | Bread, pita, whole wheat, 100%, toasted |
| 51204010 | Bread, wheat germ |
| 51204020 | Bread, wheat germ, toasted |
| 51207010 | Bread, sprouted wheat |
| 51207020 | Bread, sprouted wheat, toasted |
| 51220000 | Roll, whole wheat, 100% |
| 51220010 | Roll, whole wheat, 100%, toasted |
| 51300110 | Bread, whole wheat, other than 100% or NS as to 100% |
| 51300120 | Bread, whole wheat, other than 100% or NS as to 100%, toasted |
| 51300180 | Bread, puri or poori (Indian puffed bread), whole wheat, other than 100% or NS as to 100%, filled with |
| 51300210 | Bread, whole wheat, NS as to 100%, with raisins |
| 51300220 | Bread, whole wheat, NS as to 100%, with raisins, toasted |
| 51301010 | Bread, wheat or cracked wheat |
| 51301020 | Bread, wheat or cracked wheat, toasted |
| 51301120 | Bread, wheat or cracked wheat, with raisins |
| 51301130 | Bread, wheat or cracked wheat, with raisins, toasted |
| 51301510 | Bread, wheat or cracked wheat, reduced calorie and/or high fiber |
| 51301520 | Bread, wheat or cracked wheat, reduced calorie and/or high fiber, toasted |
| 51301600 | Bread, pita, whole wheat, other than 100% or NS as to 100% |
| 51301610 | Bread, pita, whole wheat, other than 100% or NS as to 100%, toasted |
| 51301620 | Bread, pita, wheat or cracked wheat |
| 51301630 | Bread, pita, wheat or cracked wheat, toasted |
| 51302010 | Bread, wheat bran |
| 51302020 | Bread, wheat bran, toasted |
| 51302050 | Bread, wheat bran, with raisins |
| 51302060 | Bread, wheat bran, with raisins, toasted |
| 51320010 | Roll, wheat or cracked wheat |
| 51320020 | Rolls, wheat or cracked wheat, toasted |
| 51320500 | Roll, whole wheat, NS as to 100% |
| 51320510 | Roll, whole wheat, NS as to 100%, toasted |
| 51401010 | Bread, rye |
| 51401020 | Bread, rye, toasted |
| 51401030 | Bread, marble rye and pumpernickel |
| 51401040 | Bread, marble rye and pumpernickel, toasted |
| 51401060 | Bread, rye, reduced calorie and/or high fiber |
| 51401070 | Bread, rye, reduced calorie and/or high fiber, toasted |
| 51404010 | Bread, pumpernickel |
| 51404020 | Bread, pumpernickel, toasted |
| 51407010 | Bread, black |
| 51407020 | Bread, black, toasted |
| 51420000 | Roll, rye |
| 51421000 | Roll, pumpernickel |
| 51421100 | Roll, pumpernickel, toasted |
| 51501010 | Bread, oatmeal |

| | |
|---|---|
| 51501020 | Bread, oatmeal, toasted |
| 51501040 | Bread, oat bran |
| 51501050 | Bread, oat bran, toasted |
| 51501060 | Bread, oat bran, reduced calorie and/or high fiber |
| 51501070 | Bread, oat bran, reduced calorie and/or high fiber, toasted |
| 51502010 | Roll, oatmeal |
| 51502020 | Roll, oatmeal, toasted |
| 51502100 | Roll, oat bran |
| 51502110 | Roll, oat bran, toasted |
| 51601010 | Bread, multigrain, toasted |
| 51601020 | Bread, multigrain |
| 51601210 | Bread, multigrain, with raisins |
| 51601220 | Bread, multigrain, with raisins, toasted |
| 51602010 | Bread, multigrain, reduced calorie and/or high fiber |
| 51602020 | Bread, multigrain, reduced calorie and/or high fiber, toasted |
| 51620000 | Roll, multigrain |
| 51620010 | Roll, multigrain, toasted |
| 51801010 | Bread, barley |
| 51802010 | Bread, triticale |
| 51802020 | Bread, triticale, toasted |
| 51803010 | Bread, buckwheat |
| 51803020 | Bread, buckwheat, toasted |
| 51804010 | Bread, soy |
| 51804020 | Bread, soy, toasted |
| 51805010 | Bread, sunflower meal |
| 51805020 | Bread, sunflower meal, toasted |
| 51806010 | Bread, rice |
| 51806020 | Bread, rice, toasted |
| 51807000 | Injera (American-style Ethiopian bread) |
| 51808000 | Bread, low gluten |
| 51808010 | Bread, low gluten, toasted |

**Mixtures containing yeast breads and rolls**
(Adjusted for bread or roll content of 50.8 to 68.4%)
[Astaxanthin] = 0.00051 to 0.0007%

| | |
|---|---|
| 42301010 | Peanut butter sandwich |
| 14640300 | Cheese spread sandwich |
| 27520110 | Bacon sandwich, with spread |
| 27500050 | Sandwich, NFS |
| 27500100 | Meat sandwich, NFS |
| 27560000 | Luncheon meat sandwich, NFS, with spread |
| 27560110 | Bologna sandwich, with spread |
| 14640000 | Cheese sandwich |
| 27560410 | Puerto Rican sandwich (Sandwich criollo) |
| 27560510 | Salami sandwich, with spread |
| 27510500 | Hamburger, plain, on bun |
| 14640100 | Cheese sandwich, grilled |
| 42302010 | Peanut butter and jelly sandwich |
| 27570310 | Hors d'oeuvres, with spread |
| 32201000 | Fried egg sandwich |
| 27510600 | Hamburger, 1 oz meat, plain, on miniature bun |
| 27510210 | Cheeseburger, plain, on bun |
| 27540170 | Chicken patty sandwich, miniature, with spread |

**Mixtures containing yeast breads and rolls**
(Adjusted for bread or roll content of 40.0 to 47.8%)
[Astaxanthin] = 0.0004 to 0.00048%

| | |
|---|---|
| 27560710 | Sausage sandwich |
| 27563010 | Meat spread or potted meat sandwich |
| 27520520 | Pork sandwich |
| 27515150 | Steak patty (breaded, fried) sandwich, with mayonnaise or salad dressing, lettuce, and tomato, on bun |
| 27510590 | Hamburger, with mayonnaise or salad dressing, on bun |
| 32204010 | Scrambled egg sandwich |
| 27560120 | Bologna and cheese sandwich, with spread |
| 27540110 | Chicken sandwich, with spread |
| 27540240 | Chicken fillet, (broiled), sandwich, on whole wheat roll, with lettuce, tomato and spread |
| 27520300 | Ham sandwich, with spread |
| 27510510 | Hamburger, with tomato and/or catsup, on bun |
| 27520410 | Cuban sandwich, (Sandwich cubano), with spread |
| 27510420 | Taco burger, on bun |
| 27550750 | Tuna salad submarine sandwich, on roll, with lettuce |
| 27520120 | Bacon and cheese sandwich, with spread |
| 27560320 | Frankfurter or hot dog, plain, on bun |
| 27560400 | Chicken frankfurter or hot dog, plain, on bun |
| 27540130 | Chicken barbecue sandwich |
| 74701000 | Tomato sandwich |
| 27520310 | Ham sandwich with lettuce and spread |
| 27510311 | Cheeseburger, 1 oz meat, plain, on miniature bun |
| 27510220 | Cheeseburger, with mayonnaise or salad dressing, on bun |
| 27520330 | Ham and egg sandwich |
| 27510530 | Hamburger, 1/4 lb meat, plain, on bun |
| 27510650 | Double hamburger (2 patties), plain, on bun |
| 27510130 | Beef barbecue submarine sandwich, on bun |
| 27510270 | Double cheeseburger (2 patties), plain, on bun |
| 27510290 | Double cheeseburger (2 patties), plain, on double-decker bun |
| 54304100 | Cracker, cheese, reduced fat |
| 58128220 | Dressing with chicken or turkey and vegetables |
| 27550100 | Fish sandwich, on bun, with cheese and spread |
| 27560670 | Sausage and cheese on English muffin |

**Mixtures containing yeast breads and rolls**
(Adjusted for bread or roll content of 30.0 to 39.6%)
[Astaxanthin] = 0.0003 to 0.0004%

| | |
|---|---|
| 27510720 | Pizzaburger (hamburger, cheese, sauce) on whole bun |
| 27510520 | Hamburger, with mayonnaise or salad dressing and tomatoes, on bun |
| 27540190 | Chicken patty sandwich, with lettuce and spread |
| 27510310 | Cheeseburger with tomato and/or catsup, on bun |
| 42303010 | Peanut butter and banana sandwich |
| 27513060 | Roast beef sandwich with bacon and cheese sauce |
| 27560340 | Frankfurter or hot dog, with catsup and/or mustard, on bun |
| 58128250 | Dressing with meat and vegetables |
| 27510000 | Beef sandwich, NFS |
| 27513010 | Roast beef sandwich |
| 27510910 | Corned beef sandwich |
| 27511010 | Pastrami sandwich |
| 27520500 | Pork, barbecue sauce, onions and dill pickles on white roll |

| | |
|---|---|
| 27520130 | Bacon, chicken, and tomato club sandwich, with lettuce and spread |
| 27520340 | Ham salad sandwich |
| 27540120 | Chicken salad or chicken spread sandwich |
| 27540320 | Turkey salad or turkey spread sandwich |
| 27510240 | Cheeseburger, 1/4 lb meat, plain, on bun |
| 27510610 | Hamburger, 1 oz meat, with tomato and/or catsup, on miniature bun |
| 14640200 | Cheese sandwich, hoagie |
| 27540310 | Turkey sandwich, with spread |
| 27520420 | Midnight sandwich, (Media noche), with spread |
| 27540260 | Chicken fillet, broiled, sandwich, on oat bran bun, with lettuce, tomato, spread |
| 27513070 | Roast beef submarine sandwich, on roll, au jus |
| 27540350 | Turkey submarine sandwich, on roll, with cheese, lettuce, tomato and spread |
| 27510630 | Hamburger, 1/4 lb meat, with mayonnaise or salad dressing, on bun |
| 27510550 | Double hamburger (2 patties), with mayonnaise or salad dressing and tomatoes, on double-decker bun |
| 27520320 | Ham and cheese sandwich, with lettuce and spread |
| 27540250 | Chicken fillet, broiled, sandwich with cheese, on whole wheat roll, with lettuce, tomato and non-may |
| 27520150 | Bacon, lettuce, and tomato sandwich with spread |
| 27510230 | Cheeseburger, with mayonnaise or salad dressing and tomatoes, on bun |
| 27520350 | Ham and cheese sandwich, with spread, grilled |
| 27510280 | Double cheeseburger (2 patties), with mayonnaise or salad dressing, on bun |
| 27540230 | Chicken patty sandwich with cheese, on wheat bun, with lettuce, tomato and spread |
| 27510570 | Hamburger, 2-1/2 oz meat, with mayonnaise or salad dressing and tomatoes, on bun |
| 27510620 | Hamburger, 1/4 lb meat, with tomato and/or catsup, on bun |
| 27560330 | Frankfurter or hot dog, with cheese, plain, on bun |
| 27515010 | Steak sandwich, plain, on roll |
| 27540140 | Chicken fillet (breaded, fried) sandwich |
| 27515030 | Steak and cheese sandwich, plain, on roll |
| 27510300 | Double cheeseburger (2 patties), with mayonnaise or salad dressing, on double-decker bun |
| 27550720 | Tuna salad sandwich |
| 27510410 | Chiliburger, on bun |
| 27540280 | Chicken fillet, broiled, sandwich with cheese, on bun, with lettuce, tomato and spread |
| 27510360 | Cheeseburger with mayonnaise or salad dressing, tomato and bacon, on bun |
| 27513050 | Roast beef sandwich with cheese |
| 32203010 | Egg salad sandwich |
| 27510660 | Double hamburger (2 patties), with mayonnaise or salad dressing, on bun |
| 27560720 | Sausage and spaghetti sauce sandwich |
| 27515070 | Steak and cheese submarine sandwich, with fried peppers and onions, on roll |
| 27513040 | Roast beef submarine sandwich, on roll, with lettuce, tomato and spread |
| 27515000 | Steak submarine sandwich, on roll, with lettuce and tomato |
| 27510540 | Double hamburger (2 patties), with tomato and/or catsup, on bun |
| 27540270 | Chicken fillet, broiled, sandwich, with lettuce, tomato, and non-mayonnaise type spread |
| 27250450 | Shrimp toast, fried |
| 27550710 | Tuna salad sandwich, with lettuce |
| 27510560 | Hamburger, 1/4 lb meat, with mayonnaise or salad dressing and tomatoes, on bun |
| 27540150 | Chicken fillet (breaded, fried) sandwich with lettuce, tomato and spread |
| 27510340 | Double cheeseburger (2 patties), with mayonnaise or salad dressing and tomatoes, on bun |

| | |
|---|---|
| 27510480 | Cheeseburger (hamburger with cheese sauce), 1/4 lb meat, with grilled onions, on rye bun |
| 27510250 | Cheeseburger, 1/4 lb meat, with mayonnaise or salad dressing, on bun |
| 27510700 | Meatball and spaghetti sauce submarine sandwich, on roll |
| 27510450 | Cheeseburger, 1/4 lb meat, with ham, on bun |
| 41901020 | Soyburger, meatless, with cheese on bun |
| 21420100 | Beef, sandwich steak (flaked, formed, thinly sliced) |
| 27510400 | Bacon cheeseburger, 1/4 lb meat, with tomato and/or catsup, on bun |
| 27510440 | Bacon cheeseburger, 1/4 lb meat, with mayonnaise or salad dressing and tomatoes, on bun |
| 27550730 | Tuna melt sandwich |
| 27560660 | Sausage griddle cake sandwich |
| 32202055 | Egg, cheese, and sausage griddle cake sandwich |
| 32202075 | Egg, cheese, and bacon griddle cake sandwich |

**Mixtures containing yeast breads and rolls**
(Adjusted for bread or roll content of 20.7 to 29.7%)
[Astaxanthin] = 0.0002 to 0.0003%

| | |
|---|---|
| 27516010 | Gyro sandwich (pita bread, beef, lamb, onion, condiments), with tomato and spread |
| 58128210 | Dressing with oysters |
| 27510670 | Double hamburger (2 patties), with mayonnaise or salad dressing and tomatoes, on bun |
| 27510330 | Double cheeseburger (2 patties), with tomato and/or catsup, on bun |
| 27510320 | Cheeseburger, 1/4 lb meat, with tomato and/or catsup, on bun |
| 27520140 | Bacon and egg sandwich |
| 27515020 | Steak and cheese submarine sandwich, on roll, with lettuce and tomato |
| 27520360 | Ham and cheese sandwich, on bun, with lettuce and spread |
| 27520530 | Pork sandwich, with gravy |
| 27510110 | Beef barbecue or Sloppy Joe, on bun |
| 27520540 | Ham and tomato club sandwich, with lettuce and spread |
| 27510350 | Cheeseburger, 1/4 lb meat, with mayonnaise or salad dressing and tomatoes, on bun |
| 27560370 | Frankfurter or hot dog with chili and cheese, on bun |
| 58310110 | Frozen breakfast, NFS (frozen meal) |
| 58310210 | Sausage and french toast (frozen meal) |
| 27510950 | Reuben sandwich (corned beef sandwich with sauerkraut and cheese), with spread |
| 27560360 | Frankfurter or hot dog, with chili, on bun |
| 27510680 | Double hamburger (2 patties, 1/4 lb meat each), with tomato and/or catsup, on bun |
| 27520370 | Hot ham and cheese sandwich, on bun |
| 27520510 | Pork barbecue or Sloppy Joe, on bun |
| 27520160 | Bacon, chicken, and tomato club sandwich, on multigrain roll with lettuce and spread |
| 27510260 | Cheeseburger, 1/4 lb meat, with mushrooms in sauce, on bun |
| 13210150 | Puerto Rican bread pudding made with evaporated milk and rum (Budin de pan) |
| 27550510 | Sardine sandwich, with lettuce and spread |
| 27510710 | Pizzaburger (hamburger, cheese, sauce) on 1/2 bun |
| 27560310 | Corny dog, with chili, on bun |
| 27515040 | Steak and cheese submarine sandwich, plain, on roll |
| 27513020 | Roast beef sandwich, with gravy |
| 27560910 | Submarine, cold cut sandwich, on bun, with lettuce |
| 27510690 | Double hamburger (2 patties, 1/4 lb meat each), with mayonnaise or salad dressing and tomatoes and/o |

| | |
|---|---|
| 27510370 | Double cheeseburger (2 patties, 1/4 lb meat each), with mayonnaise or salad dressing, on bun |
| 27510390 | Double bacon cheeseburger (2 patties, 1/4 lb meat each), on bun |
| 13210190 | Pudding, Mexican bread (Capirotada), lower fat |

**Mixtures containing yeast breads and rolls**
(Adjusted for bread or roll content of 11.4 to 19.7%)
[Astaxanthin] = 0.00011 to 0.0002%

| | |
|---|---|
| 27513030 | Roast beef sandwich dipped in egg, fried, with gravy and spread |
| 27510430 | Double bacon cheeseburger (2 patties, 1/4 lb meat each), with mayonnaise or salad dressing and tomato |
| 13210180 | Pudding, Mexican bread (Capirotada) |
| 27540330 | Turkey sandwich, with gravy |
| 27520390 | Ham and cheese submarine sandwich, on multigrain roll, with lettuce, tomato and spread |
| 27515050 | Fajita-style beef sandwich with cheese, on pita bread, with lettuce and tomato |
| 27540200 | Fajita-style chicken sandwich with cheese, on pita bread, with lettuce and tomato |
| 55310100 | Bread fritters, Puerto Rican style (Torrejas, Galician fritters) |
| 27510380 | Triple cheeseburger (3 patties, 1/4 lb meat each), with mayonnaise or salad dressing and tomatoes, o |
| 27214600 | Creamed dried beef on toast |
| 32105190 | Egg casserole with bread, cheese, milk and meat |
| 13210110 | Pudding, bread |
| 27250150 | Tuna loaf |
| 27319010 | Stuffed green pepper, Puerto Rican style (Pimiento relleno) |

**Mixtures containing yeast breads and rolls**
(Adjusted for bread or roll content of 6.2 to 10.7%)
[Astaxanthin] = 0.00006 to 0.0001%

| | |
|---|---|
| 27250080 | Salmon loaf |
| 72125260 | Spinach and cheese casserole |
| 27351020 | Codfish salad, Puerto Rican style (Gazpacho de bacalao) |
| 27246500 | Meat loaf made with chicken or turkey |
| 74203010 | Tomatoes, NS as to form, scalloped |
| 74203011 | Tomatoes, from fresh, scalloped |
| 28145100 | Turkey with dressing, gravy, vegetable and fruit (diet frozen meal) |
| 27214100 | Meat loaf made with beef |
| 27235000 | Meat loaf made with venison / deer |
| 27260010 | Meat loaf, NS as to type of meat |
| 27260080 | Meat loaf made with beef and pork |
| 27260090 | Meat loaf made with beef, veal and pork |
| 27260510 | Liver dumpling |
| 32301100 | Garlic egg soup, Puerto Rican style (Sopa de ajo) |
| 58305010 | Mosticolli with meatballs, sauce, bread (frozen meal) |
| 72116140 | Caesar salad (with romaine) |
| 27214110 | Meat loaf made with beef, with tomato-based sauce |
| 27260100 | Meat loaf made with beef and pork, with tomato-based sauce |
| 28141050 | Chicken patty parmigiana, breaded, with vegetable (diet frozen meal) |

**Mixtures containing yeast breads and rolls**
(Adjusted for bread or roll content of 0.19 to 5.4%)
[Astaxanthin] = 0.000002 to 0.00005%

| | |
|---|---|
| 41306000 | Loaf, lentil |
| 27260050 | Meatballs, with breading, NS as to type of meat, with gravy |
| 27550000 | Fish sandwich, on bun, with spread |
| 27230010 | Lamb or mutton loaf |
| 27550110 | Crab cake sandwich, on bun |
| 58162090 | Stuffed pepper, with meat |
| 58162120 | Stuffed pepper, with rice, meatless |
| 58131310 | Ravioli, meat-filled, no sauce |
| 58131320 | Ravioli, meat-filled, with tomato sauce or meat sauce |
| 58131100 | Ravioli, NS as to filling, no sauce |
| 58131110 | Ravioli, NS as to filling, with tomato sauce |

**Beverages and Beverage Bases**

**Bottled, Enhanced, and Carbonated Water**
[Astaxanthin] = 0.0002%

| | |
|---|---|
| 92410110 | Carbonated water, sweetened |
| 92410210 | Carbonated water, unsweetened |
| 92410250 | Carbonated water, sugar-free |
| 94100100 | Water, bottled, unsweetened |
| 94210100 | Propel Fitness Water |

**Mixtures containing bottled, enhanced or carbonated water**
(Adjusted for bottled, enhanced or carbonated water content of 57.0 to 74.5%)
[Astaxanthin] = 0.00011 to 0.00015%

| | |
|---|---|
| 93301060 | Gin and Tonic |
| 93301080 | High ball |
| 93301150 | Tom Collins |
| 93301230 | Sloe gin fizz |
| 93301330 | Gin Rickey |
| 93301600 | Gin fizz |
| 13120800 | Ice cream soda, flavors other than chocolate |
| 93301170 | Bourbon and soda |
| 93301280 | Singapore Sling |
| 13120810 | Ice cream soda, chocolate |

**Mixtures containing bottled, enhanced or carbonated water**
(Adjusted for bottled, enhanced or carbonated water content of 18.1 to 40.1%)
[Astaxanthin] = 0.00004 to 0.00008%

| | |
|---|---|
| 93405000 | Wine spritzer |
| 92431000 | Carbonated juice drink, NS as to type of juice |
| 92433000 | Carbonated noncitrus juice drink |
| 92432000 | Carbonated citrus juice drink |
| 93404500 | Sangria |
| 93301270 | Fruit punch, alcoholic |
| 93404600 | Sangria, Puerto Rican style |

**Carbonated Beverages**
[Astaxanthin] = 0.0002%

| | |
|---|---|
| 92400000 | Soft drink, NFS |
| 92400100 | Soft drink, NFS, sugar-free |
| 92410310 | Soft drink, cola-type |
| 92410315 | Soft drink, cola type, reduced sugar |
| 92410320 | Soft drink, cola-type, sugar-free |
| 92410330 | Soft drink, cola-type, with higher caffeine |
| 92410340 | Soft drink, cola-type, decaffeinated |
| 92410350 | Soft drink, cola-type, decaffeinated, sugar-free |
| 92410360 | Soft drink, pepper-type |
| 92410370 | Soft drink, pepper-type, sugar-free |
| 92410390 | Soft drink, pepper-type, decaffeinated |
| 92410400 | Soft drink, pepper-type, decaffeinated, sugar-free |
| 92410410 | Cream soda |
| 92410420 | Cream soda, sugar-free |
| 92410510 | Soft drink, fruit-flavored, caffeine free |
| 92410520 | Soft drink, fruit-flavored, sugar free, caffeine free |
| 92410550 | Soft drink, fruit flavored, caffeine containing |
| 92410560 | Soft drink, fruit flavored, caffeine containing, sugar-free |
| 92410610 | Ginger ale |
| 92410620 | Ginger ale, sugar-free |
| 92410710 | Root beer |
| 92410720 | Root beer, sugar-free |
| 92410810 | Chocolate-flavored soda |
| 92410820 | Chocolate-flavored soda, sugar-free |
| 92411510 | Cola with fruit or vanilla flavor |
| 92411520 | Cola with chocolate flavor |
| 92411610 | Cola with fruit or vanilla flavor, sugar-free |
| 92411620 | Cola with chocolate flavor, sugar-free |
| 92416010 | Mavi drink |
| 92417010 | Soft drink, ale type |

**Mixtures containing carbonated beverages**
(Adjusted for carbonated beverage content of 26.1 to 74.0%)
[Astaxanthin] = 0.00005 to 0.00015%

| | |
|---|---|
| 92432000 | Carbonated citrus juice drink |
| 92431000 | Carbonated juice drink, NS as to type of juice |
| 92433000 | Carbonated noncitrus juice drink |
| 92510720 | Fruit punch, made with fruit juice and soda |
| 92510730 | Fruit punch, made with soda, fruit juice, and sherbet or ice cream |

**Energy, Sports and Isotonic Drinks**
[Astaxanthin] = 0.0002%

| | |
|---|---|
| 11631000 | High calorie beverage, canned or powdered, reconstituted |
| 92553000 | Fruit-flavored thirst quencher beverage, low calorie |
| 92560000 | Fruit-flavored thirst quencher beverage |
| 92570100 | Fluid replacement, electrolyte solution |
| 92570500 | Fluid replacement, 5% glucose in water |
| 92650000 | Red Bull Energy Drink |
| 92651000 | Energy drink |

**Non-reconstituted energy, sport or isotonic drink**
(Adjusted for not being reconstituted, 15g of powder to 240mL of water)
[Astaxanthin] = 0.0032%

| | |
|---|---|
| 92900300 | Fruit-flavored thirst quencher beverage, dry concentrate, not reconstituted |

**Non-Milk Based Meal Replacements**
[Astaxanthin] = 0.0002%

| | |
|---|---|
| 41430200 | Meal replacement or supplement, soy- and milk-base, powder, reconstituted with water |
| 41440010 | Meal replacement or supplement, liquid, soy-base, high protein |
| 41440020 | Ensure with fiber, liquid |
| 41440050 | Ensure Plus liquid nutrition |
| 41440100 | Meal replacement or supplement, liquid, soy-based |

**Non-reconstituted non-milk based meal replacements**
(Adjusted for not being reconstituted, 15g of powder to 240mL of water)
[Astaxanthin] = 0.0032%

| | |
|---|---|
| 41430000 | Protein powder, NFS |
| 41430010 | Protein supplement, powdered |
| 41430310 | Protein diet powder with soy and casein |

**<u>Cereals and Cereal Products</u>**

**Cereal and Energy Bars**
[Astaxanthin] = 0.0013%

| | |
|---|---|
| 41435110 | High protein bar, candy-like, soy and milk base |
| 53540000 | Breakfast bar, NFS |
| 53540200 | Breakfast bar, cereal crust with fruit filling, lowfat |
| 53540250 | Breakfast bar, cereal crust with fruit filling, fat free |
| 53540500 | Breakfast bar, date, with yogurt coating |
| 53540600 | Milk 'n Cereal bar |
| 53541200 | Meal replacement bar |
| 53542100 | Granola bar, oats, sugar, raisins, coconut |
| 53542200 | Granola bar, oats, fruit and nuts, lowfat |
| 53542210 | Granola bar, nonfat |
| 53543100 | Granola bar, peanuts, oats, sugar, wheat germ |
| 53544200 | Granola bar, chocolate-coated |
| 53544210 | Granola bar, with coconut, chocolate-coated |
| 53544220 | Granola bar with nuts, chocolate-coated |
| 53544250 | Granola bar, coated with non-chocolate coating |
| 53544300 | Granola bar, high fiber, coated with non-chocolate yogurt coating |
| 53544400 | Granola bar, with rice cereal |
| 53544450 | PowerBar (fortified high energy bar) |

**Instant and Hot Cereals**
[Astaxanthin] = 0.0002%

| | |
|---|---|
| 56200300 | Cereal, cooked, NFS |
| 56200350 | Cereal, cooked, instant, NS as to grain |
| 56202960 | Oatmeal, cooked, NS as to regular, quick or instant; NS as to fat added in cooking |
| 56202970 | Oatmeal, cooked, quick (1 or 3 minutes), NS as to fat added in cooking |

| | |
|---|---|
| 56202980 | Oatmeal, cooked, regular, NS as to fat added in cooking |
| 56203000 | Oatmeal, cooked, NS as to regular, quick or instant, fat not added in cooking |
| 56203010 | Oatmeal, cooked, regular, fat not added in cooking |
| 56203020 | Oatmeal, cooked, quick (1 or 3 minutes), fat not added in cooking |
| 56203030 | Oatmeal, cooked, instant, fat not added in cooking |
| 56203040 | Oatmeal, cooked, NS as to regular, quick, or instant, fat added in cooking |
| 56203050 | Oatmeal, cooked, regular, fat added in cooking |
| 56203060 | Oatmeal, cooked, quick (1 or 3 minutes), fat added in cooking |
| 56203070 | Oatmeal, cooked, instant, fat added in cooking |
| 56203080 | Oatmeal, cooked, instant, NS as to fat added in cooking |
| 56203110 | Oatmeal with maple flavor, cooked |
| 56203200 | Oatmeal with fruit, cooked |
| 56203210 | Oatmeal, NS as to regular, quick, or instant, made with milk, fat not added in cooking |
| 56203220 | Oatmeal, NS as to regular, quick, or instant, made with milk, fat added in cooking |
| 56203230 | Oatmeal, NS as to regular, quick, or instant, made with milk, NS as to fat added in cooking |
| 56203540 | Oatmeal, made with evaporated milk and sugar, Puerto Rican style |
| 56203600 | Oatmeal, multigrain, cooked, NS as to fat added in cooking |
| 56203610 | Oatmeal, multigrain, cooked, fat not added in cooking |
| 56203620 | Oatmeal, multigrain, cooked, fat added in cooking |
| 56206970 | Wheat, cream of, cooked, quick, NS as to fat added in cooking |
| 56206980 | Wheat, cream of, cooked, regular, NS as to fat added in cooking |
| 56206990 | Wheat, cream of, cooked, NS as to regular, quick, or instant, NS as to fat added in cooking |
| 56207000 | Wheat, cream of, cooked, NS as to regular, quick, or instant, fat not added in cooking |
| 56207010 | Wheat, cream of, cooked, regular, fat not added in cooking |
| 56207020 | Wheat, cream of, cooked, quick, fat not added in cooking |
| 56207030 | Wheat, cream of, cooked, instant, fat not added in cooking |
| 56207040 | Wheat, cream of, cooked, made with milk |
| 56207050 | Wheat, cream of, cooked, made with milk and sugar, Puerto Rican style |
| 56207060 | Wheat, cream of, cooked, instant, fat added in cooking |
| 56207070 | Wheat, cream of, cooked, instant, NS as to fat added in cooking |
| 56207080 | Wheat, cream of, cooked, NS as to regular, quick, or instant, fat added in cooking |
| 56207190 | Whole wheat cereal, cooked, NS as to fat added in cooking |
| 56207200 | Whole wheat cereal, cooked, fat not added in cooking |
| 56207210 | Whole wheat cereal, cooked, fat added in cooking |
| 56207220 | Wheat, cream of, cooked, regular, fat added in cooking |
| 56207230 | Wheat, cream of, cooked, quick, fat added in cooking |
| 56207300 | Whole wheat cereal, wheat and barley, cooked, fat not added in cooking |
| 56207310 | Wheat hearts, cooked, fat not added in cooking |
| 56207330 | Whole wheat cereal, wheat and barley, cooked, fat added in cooking |
| 56207340 | Whole wheat cereal, wheat and barley, cooked, NS as to fat added in cooking |
| 56207350 | Wheat cereal, chocolate flavored, cooked, made with milk |
| 56207360 | Wheat cereal, chocolate flavored, cooked, fat not added in cooking |
| 56207370 | Wheat cereal, chocolate flavored, cooked, NS as to fat added in cooking |
| 56208500 | Oat bran cereal, cooked, fat not added in cooking |
| 56208510 | Oat bran cereal, cooked, fat added in cooking |
| 56208520 | Oat bran cereal, cooked, NS as to fat added in cooking |
| 56208530 | Oat bran cereal, cooked, made with milk, fat not added in cooking |
| 56208540 | Oat bran cereal, cooked, made with milk, fat added in cooking |
| 56208550 | Oat bran cereal, cooked, made with milk, NS as to fat added in cooking |
| 56209000 | Rye, cream of, cooked |
| 56210000 | Nestum cereal |

**Ready-to-Eat Cereals**
[Astaxanthin] = 0.0033%

| | |
|---|---|
| 57137000 | Corn Puffs |
| 57301500 | Kashi, Puffed |
| 57306100 | Malt-O-Meal Puffed Rice |
| 57306120 | Malt-O-Meal Puffed Wheat |
| 57307500 | Millet, puffed |
| 57340000 | Rice, puffed |
| 57416000 | Wheat, puffed, plain |
| 57416010 | Wheat, puffed, presweetened with sugar |

[Astaxanthin] = 0.0017%

| | |
|---|---|
| 57100100 | Cereal, ready-to-eat, NFS |
| 57101000 | All-Bran |
| 57101020 | All-Bran with Extra Fiber |
| 57101500 | Almond Delight |
| 57102000 | Alpen |
| 57103000 | Alpha-Bits |
| 57103020 | Alpha-bits with marshmallows |
| 57103050 | Amaranth Flakes |
| 57103100 | Apple Cinnamon Cheerios |
| 57103400 | Apple Cinnamon Oh's Cereal |
| 57103450 | Apple Cinnamon Rice Krispies |
| 57104000 | Apple Jacks |
| 57105000 | Apple Raisin Crisp |
| 57106000 | Banana Frosted Flakes |
| 57106200 | Batman |
| 57106250 | Berry Berry Kix |
| 57106300 | Bigg Mixx |
| 57106500 | Bill and Ted's Excellent Adventure |
| 57106530 | Blueberry Morning, Post |
| 57107000 | Booberry |
| 57109000 | Body Buddies, natural fruit flavor |
| 57110000 | All-Bran Bran Buds, Kellogg's (formerly Bran Buds) |
| 57111000 | Bran Chex |
| 57111300 | Bran News |
| 57112000 | Branola |
| 57112500 | Breakfast with Barbie |
| 57113000 | Buc Wheats |
| 57113300 | Bunuelitos |
| 57114000 | C-3PO's |
| 57115000 | C.W. Post Plain |
| 57116000 | C.W. Post w/raisins |
| 57116100 | Cabbage Patch |
| 57117000 | Cap'n Crunch |
| 57117500 | Cap'n Crunch's Christmas Crunch |
| 57118000 | Cap'n Crunch's Choco Crunch |
| 57119000 | Cap'n Crunch's Crunch Berries |
| 57119500 | Cap'n Crunch's Deep Sea Crunch |
| 57120000 | Cap'n Crunch's Peanut Butter Crunch |
| 57123000 | Cheerios |
| 57124000 | Chex cereal, NFS |
| 57124200 | Chocolate flavored frosted puffed corn cereal |
| 57124500 | Cinnamon Grahams, General Mills |

| | |
|---|---|
| 57125000 | Cinnamon Toast Crunch |
| 57125900 | Honey Nut Clusters (formerly called Clusters) |
| 57126000 | Cocoa Krispies |
| 57126500 | Cocoa Blasts, Quaker |
| 57127000 | Cocoa Pebbles |
| 57128000 | Cocoa Puffs |
| 57128880 | Common Sense Oat Bran, plain |
| 57128900 | Common Sense Oat Bran, with raisins |
| 57130000 | Cookie-Crisp |
| 57131000 | Crunchy Corn Bran, Quaker |
| 57132000 | Corn Chex |
| 57134000 | Corn flakes, NFS |
| 57134090 | Corn flakes, low sodium |
| 57135000 | Corn flakes, Kellogg |
| 57138000 | Total Corn Flakes |
| 57139000 | Count Chocula |
| 57141000 | Cracker Jack |
| 57144000 | Crisp Crunch |
| 57148000 | Crispix |
| 57148500 | Crispy Brown Rice Cereal |
| 57151000 | Crispy Rice |
| 57152000 | Crispy Wheats'n Raisins |
| 57152100 | Croonchy Stars |
| 57201200 | Dairy Crisp w/strawberries |
| 57201700 | Dino Pebbles |
| 57202100 | Donkey Kong |
| 57203100 | Donkey Kong Jr. |
| 57204100 | Donutz Cereal |
| 57205100 | Donutz Cereal, Chocolate |
| 57205250 | Double Chex |
| 57205260 | Double Dip Crunch, Kellogg's |
| 57205300 | E.T. Cereal |
| 57206000 | Familia |
| 57206700 | Fiber One |
| 57206800 | Fiber 7 Flakes, Health Valley |
| 57210100 | 40+ Bran Flakes |
| 57211000 | Frankenberry |
| 57212100 | French Toast Crunch, General Mills |
| 57213000 | Froot Loops |
| 57213800 | Frosted Bran, Kellogg's |
| 57213850 | Frosted Cheerios |
| 57214100 | Frosted Wheat Bites |
| 57215000 | Frosty O's |
| 57216000 | Frosted rice, NFS |
| 57217000 | Frosted Rice Krinkles |
| 57218000 | Frosted Rice Krispies |
| 57219000 | Fruit & Fibre (fiber), NFS |
| 57220000 | Fruit & Fibre (fiber) with apples and cinnamon |
| 57221000 | Fruit & Fibre (fiber) with dates, raisins, and walnuts |
| 57221250 | Fruit & Fibre (fiber) harvest medley |
| 57221500 | Fruit & Fibre (fiber) tropical fruit w/oat clusters |
| 57221600 | Fruit & Fibre (fiber) with peaches, raisins, almonds and oat clusters |
| 57221700 | Fruit Rings, NFS |
| 57221800 | Fruit Whirls |
| 57222000 | Fruitful Bran |
| 57222500 | Fruit Wheats |

| | |
|---|---|
| 57223000 | Fruity Pebbles |
| 57223200 | Fruity Yummy Mummy cereal |
| 57223800 | G.I. Joe Action Stars |
| 57224000 | Golden Grahams |
| 57225000 | Golden Harvest Proteinola |
| 57227000 | Granola, NFS |
| 57228000 | Granola, homemade |
| 57230000 | Grape-Nuts |
| 57231000 | Grape-Nut Flakes |
| 57231500 | Gremlins |
| 57232000 | Halfsies |
| 57232100 | Healthy Choice Almond Crunch with raisins, Kellogg's |
| 57232110 | Healthy Choice Multi-Grain Squares, Kellogg's |
| 57232120 | Healthy Choice Multi-Grain Flakes, Kellogg's |
| 57233000 | Heartland Natural Cereal, plain |
| 57234000 | Heartland Natural Cereal, with raisins |
| 57235000 | Heartland Natural Cereal, with coconut |
| 57235500 | Heartwise, plain |
| 57235600 | Heartwise, with fruit nuggets |
| 57235650 | Hidden Treasures, General Mills |
| 57237000 | Honey Bran |
| 57237100 | Honey Bunches of Oats |
| 57237300 | Honey Bunches of Oats with Almonds, Post |
| 57238000 | Honeycomb, plain |
| 57239000 | Honeycomb, strawberry |
| 57239100 | Honey Crunch Corn Flakes, Kellogg's |
| 57240000 | Honey Graham Chex |
| 57240100 | Honey Nut Chex |
| 57241000 | Honey Nut Cheerios |
| 57243000 | Honey Smacks |
| 57243500 | Horizon |
| 57243600 | Hot Wheels |
| 57243870 | Jenny O's |
| 57243900 | Jetsons |
| 57301100 | Kaboom |
| 57301700 | Kenmei Rice Bran |
| 57302100 | King Vitaman |
| 57303100 | Kix |
| 57304100 | Life (plain and cinnamon) |
| 57305100 | Lucky Charms |
| 57305150 | Frosted oat cereal with marshmallows |
| 57305170 | Malt-O-Meal Coco-Roos |
| 57305180 | Malt-O-Meal Corn Bursts |
| 57305200 | Malt-O-Meal Crispy Rice |
| 57305500 | Malt-O-Meal Honey and Nut Toasty O's |
| 57305600 | Malt-O-Meal Marshmallow Mateys |
| 57306500 | Malt-O-Meal Golden Puffs (formerly Sugar Puffs) |
| 57306700 | Malt-O-Meal Toasted Oat Cereal |
| 57306800 | Malt-O-meal Tootie Fruities |
| 57307100 | Fruity Marshmallow Krispies (formerly called Marshmallow Krispies) |
| 57307150 | Marshmallow Safari, Quaker |
| 57307550 | Mini Buns Cereal (cinnamon) |
| 57308000 | Morning Funnies |
| 57308100 | Most |
| 57308250 | Mr. T |
| 57308300 | Multi Bran Chex |

| | |
|---|---|
| 57308400 | Multi Grain Cheerios |
| 57308410 | Multi-Grain Cheerios Plus |
| 57311600 | Nintendo |
| 57311700 | Nu System Cuisine Toasted Grain Circles |
| 57311790 | Nut and Honey crunch biscuits |
| 57311800 | Nut and Honey Crunch (flakes) |
| 57311900 | Nutrific Oatmeal Flakes |
| 57312100 | Nutri-Grain Biscuits, Whole Grain Shredded Wheat Cereal |
| 57313000 | Nutri-Grain Corn |
| 57313900 | Nutri-Grain Raisin Bran |
| 57315000 | Nutri-Grain Golden Wheat (formerly Nutri-Grain Wheat) |
| 57316100 | Nutri-Grain Almond Raisin |
| 57316200 | Nutty Nuggets, Ralston Purina |
| 57316300 | Oat Bran Flakes, Health Valley |
| 57316350 | Oat Bran Options |
| 57316360 | Oatbake, Honey Bran |
| 57316370 | Oatbake, Raisin Nut |
| 57316400 | Oatmeal Crisp |
| 57316700 | Oh's, Crunchy Nut |
| 57316710 | Oh's, Honey Graham |
| 57316750 | Oh's, Fruitangy, Quaker |
| 57316800 | O.J.'s |
| 57317000 | Oat flakes, fortified |
| 57317200 | Oat Flakes, Post |
| 57322500 | Oreo O's cereal, Post |
| 57323000 | Sweet Crunch, Quaker (formerly called Popeye) |
| 57323050 | Sweet Puffs, Quaker |
| 57323200 | Pop Tarts Crunch Cereal |
| 57325000 | Product 19 |
| 57327450 | Quaker Oat Bran Cereal |
| 57327600 | Quaker Rice Bran |
| 57328000 | Quisp |
| 57328500 | Rainbow Brite |
| 57330500 | Raisin Bran, Nutri System |
| 57332000 | Raisin Bran, Ralston Purina |
| 57332300 | Super Raisin Bran, New Morning |
| 57333000 | Raisin Grape-Nuts |
| 57334000 | Raisin Life |
| 57335530 | Razzle Dazzle Rice Krispies |
| 57335550 | Reese's Peanut Butter Puffs cereal |
| 57336000 | Rice Chex |
| 57337000 | Rice Flakes, NFS |
| 57339000 | Rice Krispies |
| 57339500 | Rice Krispies Treats Cereal (Kellogg's) |
| 57340200 | Ripple Crisp Golden Corn |
| 57340210 | Ripple Crisp Honey Bran, General Mills |
| 57340500 | Rocky Road |
| 57340900 | S.W. Graham |
| 57342000 | Slimer! & Ghostbusters |
| 57342500 | S'mores Crunch |
| 57343000 | Smurf Magic Berries |
| 57344000 | Special K |
| 57344050 | Spider-Man, Ralston |
| 57344100 | Sprinkle Spangles |
| 57345000 | Strawberry Krispies |
| 57346000 | Strawberry Shortcake |

| | |
|---|---|
| 57346200 | Sun Crunchers Cereal, General Mills |
| 57346500 | Toasted Oatmeal, Honey Nut (Quaker) |
| 57347000 | Corn Pops |
| 57347500 | Strawberry Squares Mini-Wheats, Kellogg's (formerly Strawberry Squares) |
| 57348000 | Frosted corn flakes, NFS |
| 57349000 | Frosted Flakes, Kellogg |
| 57349010 | Cocoa Frosted Flakes, Kellogg's |
| 57350000 | Frosted Flakes, Ralston Purina |
| 57352000 | Sugar-Sparkled Flakes |
| 57353000 | Sugar-Sparkled Rice Krinkles |
| 57354000 | Sun Flakes |
| 57355000 | Golden Crisp (Formerly called Super Golden Crisp) |
| 57401100 | Toasted oat cereal |
| 57402000 | Team |
| 57402250 | Teddy Grahams Breakfast Bears |
| 57402500 | Teenage Mutant Ninja Turtles |
| 57402600 | Temptations, French Vanilla Almond, Kellogg's |
| 57402610 | Temptations, Honey Roasted Pecan, Kellogg's |
| 57402750 | Tiny Toon Adventures |
| 57403100 | Toasties, Post |
| 57404100 | Malt-O-Meal Toasty O's |
| 57406100 | Total |
| 57406200 | Triples |
| 57407100 | Trix |
| 57408100 | Uncle Sam's Hi Fiber Cereal |
| 57409100 | Waffle Crisp, Post |
| 57410000 | Weetabix Whole Wheat Cereal |
| 57411000 | Wheat Chex |
| 57415000 | Wheat'n Raisin Chex |
| 57417500 | Shredded Wheat with Oat Bran |
| 57418000 | Wheaties |
| 57418200 | Wheaties, Honey Frosted (formerly Wheaties Honey Gold) |

[Astaxanthin] = 0.0009%

| | |
|---|---|
| 57103500 | Apple Cinnamon Squares Mini-Wheats, Kellogg's (formerly Apple Cinnamon Squares) |
| 57106050 | Banana Nut Crunch Cereal (Post) |
| 57106100 | Basic 4 |
| 57143000 | Cracklin' Oat Bran |
| 57207000 | Bran Flakes, NFS (formerly 40% Bran Flakes, NFS) |
| 57208000 | Complete Wheat Bran Flakes, Kellogg's (formerly 40% Bran Flakes) |
| 57209000 | Natural Bran Flakes, Post (formerly called 40% Bran Flakes, Post) |
| 57214000 | Frosted Mini-Wheats |
| 57229000 | Granola, lowfat, Kellogg's |
| 57229500 | Granola with Raisins, lowfat, Kellogg's |
| 57231200 | Great Grains, Raisin, Date, and Pecan Whole Grain Cereal, Post |
| 57231250 | Great Grains Double Pecan Whole Grain Cereal, Post |
| 57241200 | Honey Nut Shredded Wheat, Post |
| 57244000 | Just Right |
| 57245000 | Just Right Fruit and Nut (formerly Just Right with raisins, dates, and nuts) |
| 57308150 | Mueslix cereal, NFS |
| 57308160 | Mueslix with raisins, walnuts, and cranberries |
| 57308170 | Muesli with raisins, peaches, and pecans |
| 57308180 | Mueslix Crispy Blend (formerly Mueslix Five Grain Muesli Cereal) |
| 57308190 | Muesli with raisins, dates, and almonds |

| | |
|---|---|
| 57308200 | Mueslix golden crunch cereal |
| 57308210 | Muesli with apples and almonds, Ralston Purina |
| 57308220 | Strawberry muesli with pecans and raisins, Ralston |
| 57308900 | Natural Muesli, Jenny's Cuisine |
| 57309100 | Nature Valley Granola, with fruit and nuts |
| 57310000 | Nature Valley Granola, with cinnamon and raisins |
| 57311000 | Nature Valley Granola, toasted oat mixture |
| 57316410 | Apple Cinnamon Oatmeal Crisp (formerly Oatmeal Crisp with Apples) |
| 57316450 | Oatmeal Crisp with Almonds |
| 57316500 | Oatmeal Raisin Crisp |
| 57318000 | 100% Bran |
| 57319000 | 100% Natural Cereal, plain, Quaker |
| 57319500 | Sun Country 100% Natural Granola, with Almonds |
| 57320500 | 100 % Natural Cereal, with oats, honey and raisins, Quaker |
| 57321000 | 100% Natural Cereal, with raisins and dates, Quaker |
| 57321500 | 100 % Natural Wholegrain Cereal with raisins, lowfat, Quaker |
| 57327500 | Quaker Oatmeal Squares (formerly Quaker Oat Squares) |
| 57329000 | Raisin bran, NFS |
| 57330000 | Raisin Bran, Kellogg |
| 57331000 | Raisin Bran, Post |
| 57332050 | Raisin Bran, Total |
| 57332100 | Raisin Nut Bran |
| 57335500 | Raisin Squares Mini-Wheats, Kellogg's (formerly Raisin Squares) |
| 57341000 | Shredded Wheat'N Bran |
| 57412000 | Wheat germ, plain |
| 57413000 | Wheat germ, with sugar and honey |
| 57417000 | Shredded Wheat, 100% |

## Chewing Gum

**Chewing Gum**
[Astaxanthin] = 0.0167%

| | |
|---|---|
| 91800100 | Chewing gum, NFS |
| 91801000 | Chewing gum, sugared |
| 91802000 | Chewing gum, uncoated, sugarless |

## Coffee and Tea

**Coffee (Instant and Regular)**
[Astaxanthin] = 0.0002%

| | |
|---|---|
| 92100000 | Coffee, NS as to type |
| 92100500 | Coffee, regular, NS as to ground or instant |
| 92101000 | Coffee, made from ground, regular |
| 92101500 | Coffee, made from ground, equal parts regular and decaffeinated |
| 92101600 | Coffee, Turkish |
| 92101610 | Coffee, espresso |
| 92101630 | Coffee, espresso, decaffeinated |
| 92101640 | Coffee, Mexican, regular, unsweetened (no milk; not cafe con leche) |
| 92101650 | Coffee, Mexican, regular, sweetened (no milk; not cafe con leche) |
| 92101660 | Coffee, Mexican, decaffeinated, unsweetened (no milk; not cafe con leche) |
| 92101670 | Coffee, Mexican, decaffeinated, sweetened (no milk; not cafe con leche) |
| 92101700 | Coffee, made from ground, regular, flavored |
| 92101800 | Coffee, Cuban |
| 92101900 | Coffee, latte |

| | |
|---|---|
| 92101910 | Coffee, latte, decaffeinated |
| 92101920 | Frappuccino, regular |
| 92101930 | Frappuccino, decaffeinated |
| 92101950 | Coffee, mocha |
| 92103000 | Coffee, made from powdered instant, regular |
| 92104000 | Coffee, made from powdered instant, 50% less caffeine |
| 92105000 | Coffee, liquid concentrate |
| 92105010 | Coffee, made from liquid concentrate |
| 92106000 | Coffee, acid neutralized, from powdered instant |
| 92111000 | Coffee, decaffeinated, NS as to ground or instant |
| 92111010 | Coffee, decaffeinated, made from ground |
| 92114000 | Coffee, decaffeinated, made from powdered instant |
| 92121010 | Coffee, made from powdered instant mix, presweetened, no whitener |
| 92130020 | Coffee, presweetened with sugar |
| 92150000 | Coffee and chicory, NS as to ground or instant |
| 92151000 | Coffee and chicory, made from powdered instant |
| 92151100 | Coffee, decaffeinated, and chicory, made from powdered instant |
| 92152000 | Coffee and chicory, made from ground |
| 92153000 | Coffee, regular, with cereal |
| 92153100 | Coffee, decaffeinated, with cereal |
| 92161000 | Cappuccino |
| 92162000 | Cappuccino, decaffeinated |
| 92191000 | Coffee, dry instant powder, NS as to regular or decaffeinated |
| 92191100 | Coffee, dry instant powder, regular |
| 92191200 | Coffee, dry instant powder, decaffeinated |
| 92191250 | Coffee, dry, acid neutralized |
| 92191500 | Coffee and chicory, dry instant powder |
| 92191520 | Coffee, decaffeinated, and chicory, dry instant powder |

**Mixtures containing coffee (instant and regular)**
(Adjusted for coffee content of 93.3 to 97.8%)
[Astaxanthin] = 0.00019 to 0.0002%

| | |
|---|---|
| 92130000 | Coffee, presweetened with sugar, pre-lightened |
| 92130010 | Coffee, pre-lightened, no sugar |
| 92121050 | Coffee and cocoa (mocha), made from powdered instant mix, with whitener and low calorie sweetener, d |
| 92192000 | Coffee and cocoa (mocha) mix, dry instant powder with whitener, presweetened |
| 92192030 | Coffee and cocoa (mocha) mix, dry instant powder with whitener and low calorie sweetener |
| 92192040 | Coffee and cocoa (mocha) mix, dry instant powder, with whitener and low calorie sweetener, decaffein |
| 92193000 | Coffee, dry instant powder, with whitener and sugar |
| 92193020 | Coffee, dry instant powder, with whitener and low calorie sweetener |
| 92121020 | Coffee and cocoa (mocha), made from powdered instant mix, with whitener, presweetened |
| 92121030 | Coffee and cocoa (mocha), made from powdered instant mix, with whitener and low calorie sweetener |
| 92121000 | Coffee, made from powdered instant mix, with whitener and sugar, instant |
| 92121040 | Coffee, made from powdered instant mix, with whitener and low calorie sweetener |

**Tea (Instant, Regular, and Specialty)**
[Astaxanthin] = 0.0002%

| | |
|---|---|
| 92301000 | Tea, NS as to type, unsweetened |
| 92301060 | Tea, NS as to type, presweetened with sugar |
| 92301080 | Tea, NS as to type, presweetened with low calorie sweetener |
| 92301100 | Tea, NS as to type, decaffeinated, unsweetened |
| 92301130 | Tea, NS as to type, presweetened, NS as to sweetener |
| 92301160 | Tea, NS as to type, decaffeinated, presweetened with sugar |
| 92301180 | Tea, NS as to type, decaffeinated, presweetened with low calorie sweetener |
| 92301190 | Tea, NS as to type, decaffeinated, presweetened, NS as to sweetener |
| 92302200 | Tea, leaf, presweetened with sugar |
| 92302300 | Tea, leaf, presweetened with low calorie sweetener |
| 92302400 | Tea, leaf, presweetened, NS as to sweetener |
| 92302500 | Tea, leaf, decaffeinated, unsweetened |
| 92302600 | Tea, leaf, decaffeinated, presweetened with sugar |
| 92302700 | Tea, leaf, decaffeinated, presweetened with low calorie sweetener |
| 92302800 | Tea, leaf, decaffeinated, presweetened, NS as to sweetener |
| 92304000 | Tea, made from frozen concentrate, unsweetened |
| 92304700 | Tea, made from frozen concentrate, decaffeinated, presweetened with low calorie sweetener |
| 92305000 | Tea, made from powdered instant, presweetened, NS as to sweetener |
| 92305010 | Tea, made from powdered instant, unsweetened |
| 92305040 | Tea, made from powdered instant, presweetened with sugar |
| 92305050 | Tea, made from powdered instant, decaffeinated, presweetened with sugar |
| 92305090 | Tea, made from powdered instant, presweetened with low calorie sweetener |
| 92305110 | Tea, made from powdered instant, decaffeinated, presweetened with low calorie sweetener |
| 92305180 | Tea, made from powdered instant, decaffeinated, unsweetened |
| 92305800 | Tea, made from powdered instant, decaffeinated, presweetened, NS as to sweetener |
| 92306000 | Tea, herbal |
| 92306020 | Tea, herbal, presweetened with sugar |
| 92306030 | Tea, herbal, presweetened with low calorie sweetener |
| 92306040 | Tea, herbal, presweetened, NS as to sweetener |
| 92306050 | Tea, made from caraway seeds |
| 92306100 | Corn beverage |
| 92306200 | Bean beverage |
| 92306610 | Tea, Russian |
| 92306700 | Tea, chamomile |

**Non-reconstituted tea (instant, regular, or specialty)**
(Adjusted for not being reconstituted, 15g of powder to 240mL of water)
[Astaxanthin] = 0.0032%

| | |
|---|---|
| 92307000 | Tea, powdered instant, unsweetened, dry |
| 92307400 | Tea, powdered instant, sweetened, NS as to sweetener, dry |

**Dairy Product Analogs**

**Imitation and Soy Milk**
[Astaxanthin] = 0.0002%

| | |
|---|---|
| 11320000 | Milk, soy, ready-to-drink, not baby's |
| 11321000 | Milk, soy, ready-to-drink, not baby's, chocolate |
| 11340000 | Milk, imitation, fluid, non-soy, sweetened, flavors other than chocolate |

000130

## Frozen Dairy Desserts and Mixes

**Frozen Yogurt**
[Astaxanthin] = 0.0004%

| | |
|---|---|
| 11459990 | Yogurt, frozen, NS as to flavor, NS as to type of milk |
| 11460000 | Yogurt, frozen, flavors other than chocolate, NS as to type of milk |
| 11460100 | Yogurt, frozen, chocolate, NS as to type of milk |
| 11460150 | Yogurt, frozen, NS as to flavor, lowfat milk |
| 11460160 | Yogurt, frozen, chocolate, lowfat milk |
| 11460170 | Yogurt, frozen, flavors other than chocolate, lowfat milk |
| 11460190 | Yogurt, frozen, NS as to flavor, nonfat milk |
| 11460200 | Yogurt, frozen, chocolate, nonfat milk |
| 11460250 | Yogurt, frozen, flavors other than chocolate, with sorbet or sorbet-coated |
| 11460300 | Yogurt, frozen, flavors other than chocolate, nonfat milk |
| 11460400 | Yogurt, frozen, chocolate, nonfat milk, with low-calorie sweetener |
| 11460410 | Yogurt, frozen, flavors other than chocolate, nonfat milk, with low-calorie sweetener |
| 11460420 | Yogurt, frozen, NS as to flavor, whole milk |
| 11460430 | Yogurt, frozen, chocolate, whole milk |
| 11460440 | Yogurt, frozen, flavors other than chocolate, whole milk |

**Mixtures containing frozen yogurt**
(Adjusted for frozen yogurt content of 24.7 to 94.1%)
[Astaxanthin] = 0.0001 to 0.0004%

| | |
|---|---|
| 11461270 | Yogurt, frozen, cone, flavors other than chocolate, lowfat milk |
| 11461280 | Yogurt, frozen, cone, chocolate, lowfat milk |
| 11461260 | Yogurt, frozen, cone, flavors other than chocolate |
| 11461250 | Yogurt, frozen, cone, chocolate |
| 11461000 | Yogurt, frozen, chocolate-coated |
| 11461100 | Yogurt, frozen, carob-coated |
| 11461200 | Yogurt, frozen, sandwich |
| 53112150 | Cake, frozen yogurt and cake layer, not chocolate, with icing |
| 53112160 | Cake, frozen yogurt and cake layer, chocolate, with icing |
| 53366000 | Pie, yogurt, frozen |

**Ice Cream and Frozen Milk Desserts**
[Astaxanthin] = 0.0004%

| | |
|---|---|
| 13110100 | Ice cream, regular, flavors other than chocolate |
| 13110120 | Ice cream, rich, flavors other than chocolate |
| 13110130 | Ice cream, rich, chocolate |
| 13110140 | Ice cream, rich, NS as to flavor |
| 13110200 | Ice cream, soft serve, flavors other than chocolate |
| 13110220 | Ice cream, soft serve, NS as to flavor |
| 13120050 | Ice cream bar or stick, not chocolate covered or cake covered |
| 13121200 | Ice cream sundae, prepackaged type, flavors other than chocolate |
| 13130300 | Light ice cream, flavors other than chocolate (formerly ice milk) |
| 13130320 | Light ice cream, no sugar added, NS as to flavor |
| 13130330 | Light ice cream, no sugar added, flavors other than chocolate |
| 13130340 | Light ice cream, no sugar added, chocolate |
| 13130590 | Light ice cream, soft serve, NS as to flavor (formerly ice milk) |
| 13130600 | Light ice cream, soft serve, flavors other than chocolate (formerly ice milk) |
| 13160150 | Milk dessert, frozen, nonfat, made with low-calorie sweetener, chocolate |

| | |
|---|---|
| 13160160 | Milk dessert, frozen, nonfat, made with low-calorie sweetener, flavors other than chocolate |
| 13160400 | Milk dessert, frozen, milk-fat free, flavors other than chocolate |
| 13160410 | Milk dessert, frozen, milk-fat free, chocolate |
| 13160420 | Fat free ice cream, NS as to flavor |
| 13161000 | Milk dessert bar, frozen, made from lowfat milk |
| 13161600 | Milk dessert bar, frozen, made from lowfat milk and low calorie sweetener |
| 13161630 | Light ice cream, bar or stick, with low-calorie sweetener, chocolate-coated (formerly ice milk) |
| 13252200 | Milk dessert or milk candy, Puerto Rican style (Dulce de leche) |

**Mixtures containing ice cream or frozen milk desserts**
(Adjusted for ice cream or frozen milk dessert content of 80.0 to 96.0%)
[Astaxanthin] = 0.0004 to 0.00032%

| | |
|---|---|
| 13140900 | Light ice cream, fudgesicle (formerly ice milk) |
| 13120730 | Ice cream cone, no topping, flavors other than chocolate |
| 13110110 | Ice cream, regular, chocolate |
| 13110210 | Ice cream, soft serve, chocolate |
| 13130310 | Light ice cream, chocolate (formerly ice milk) |
| 13130610 | Light ice cream, soft serve, chocolate (formerly ice milk) |
| 13142000 | Milk dessert bar or stick, frozen, with coconut |
| 13122100 | Ice cream pie, no crust |
| 13140110 | Light ice cream, bar or stick, chocolate covered, with nuts (formerly ice milk) |
| 13130100 | Light ice cream, NFS (formerly ice milk) |
| 13120720 | Ice cream cone, chocolate covered or dipped, flavors other than chocolate |
| 13120700 | Ice cream cone with nuts, flavors other than chocolate |
| 13110000 | Ice cream, NFS |
| 13126000 | Ice cream, fried |

**Mixtures containing ice cream or frozen milk desserts**
(Adjusted for ice cream or frozen milk dessert content of 63.0 to 79.0%)
[Astaxanthin] =0.00025 to 0.00032%

| | |
|---|---|
| 13120100 | Ice cream bar or stick, chocolate covered |
| 13140100 | Light ice cream, bar or stick, chocolate-coated (formerly ice milk) |
| 13120140 | Ice cream bar or stick, chocolate ice cream, chocolate covered |
| 13120710 | Ice cream cone, chocolate covered, with nuts, flavors other than chocolate |
| 13120500 | Ice cream sandwich |
| 13120550 | Ice cream cookie sandwich |
| 13135000 | Light ice cream, sandwich (formerly ice milk) |
| 13161500 | Milk dessert sandwich bar, frozen, made from lowfat milk |
| 13140660 | Light ice cream, sundae, soft serve, chocolate or fudge topping (without whipped cream) (formerly ice milk) |
| 13140670 | Light ice cream, sundae, soft serve, fruit topping (without whipped cream) (formerly ice milk) |
| 13140680 | Light ice cream, sundae, soft serve, not fruit or chocolate topping (without whipped cream) (formerly ice milk) |
| 13120130 | Ice cream bar or stick, rich ice cream, chocolate covered, with nuts |
| 13120121 | Ice cream bar or stick, rich ice cream, thick chocolate covering |
| 13120110 | Ice cream bar or stick, chocolate or caramel covered, with nuts |
| 13120120 | Ice cream bar or stick, rich chocolate ice cream, thick chocolate covering |

**Mixtures containing ice cream or frozen milk desserts**
(Adjusted for ice cream or frozen milk dessert content of 40.9 to 57.0%)
[Astaxanthin] = 0.00016 to 0.00023%

| | |
|---|---|
| 13121400 | Ice cream sundae, not fruit or chocolate topping, with whipped cream |
| 13120400 | Ice cream bar or stick with fruit |
| 13121000 | Ice cream sundae, NS as to topping, with whipped cream |
| 13140700 | Light ice cream, creamsicle or dreamsicle (formerly ice milk) |
| 13121100 | Ice cream sundae, fruit topping, with whipped cream |
| 13121300 | Ice cream sundae, chocolate or fudge topping, with whipped cream |
| 13140600 | Light ice cream, sundae, soft serve, chocolate or fudge topping, with whipped cream (formerly ice milk) |
| 13140630 | Light ice cream, sundae, soft serve, fruit topping, with whipped cream (formerly ice milk) |
| 13120740 | Ice cream cone, no topping, NS as to flavor |
| 13170000 | Baked Alaska |
| 13122500 | Ice cream pie, with cookie crust, fudge topping, and whipped cream |
| 13140650 | Light ice cream, sundae, soft serve, not fruit or chocolate topping, with whipped cream (formerly ice milk) |
| 13121500 | Ice cream sundae, fudge topping, with cake, with whipped cream |
| 13120810 | Ice cream soda, chocolate |

**Mixtures containing ice cream or frozen milk desserts**
(Adjusted for ice cream or frozen milk dessert content of 20.6 to 38.0%)
[Astaxanthin] = 0.00008 to 0.00015%

| | |
|---|---|
| 13161520 | Milk dessert sandwich bar, frozen, with low-calorie sweetener, made from lowfat milk |
| 53112100 | Cake, ice cream and cake roll, not chocolate |
| 53112000 | Cake, ice cream and cake roll, chocolate |
| 53430300 | Crepe, dessert type, ice cream-filled |
| 13120300 | Ice cream bar, cake covered |
| 91611050 | Ice pop filled with ice cream, all flavor varieties |
| 13120800 | Ice cream soda, flavors other than chocolate |
| 92510730 | Fruit punch, made with soda, fruit juice, and sherbet or ice cream |

**Hard Candy**

**Hard Candy**
[Astaxanthin] = 0.0033%

| | |
|---|---|
| 91700010 | Candy, NFS |
| 91718000 | Honey-combed hard candy with peanut butter |
| 91718050 | Honey-combed hard candy with peanut butter, chocolate covered |
| 91745020 | Hard candy |
| 91745040 | Butterscotch hard candy |
| 91770000 | Dietetic or low calorie candy, NFS |
| 91770020 | Dietetic or low calorie hard candy |

Mints
[Astaxanthin] = 0.025%

| | |
|---|---|
| 91770050 | Dietetic or low calorie mints |

## Milk Products

**Dry Milks**
[Astaxanthin] = 0.017%

| | |
|---|---|
| 11120000 | Milk, dry, reconstituted, NFS |
| 11121100 | Milk, dry, reconstituted, whole |
| 11121210 | Milk, dry, reconstituted, lowfat |
| 11121300 | Milk, dry, reconstituted, nonfat |

**Mixtures containing dry milks**
(Adjusted for dry milk content of 10.3 to 26.0%)
[Astaxanthin] = 0.00018 to 0.0004%

| | |
|---|---|
| 13250200 | Mousse, chocolate, lowfat, reduced calorie, prepared from dry mix, water added |
| 13142000 | Milk dessert bar or stick, frozen, with coconut |
| 13130310 | Light ice cream, chocolate (formerly ice milk) |
| 13130610 | Light ice cream, soft serve, chocolate (formerly ice milk) |
| 13140550 | Light ice cream, cone, chocolate (formerly ice milk) |

**Mixtures containing dry milks**
(Adjusted for dry milk content of 5.03 to 9.1%)
[Astaxanthin] = 0.00008 to 0.00016%

| | |
|---|---|
| 13110110 | Ice cream, regular, chocolate |
| 13110210 | Ice cream, soft serve, chocolate |
| 53205750 | Cookie, carob |
| 13120770 | Ice cream cone, no topping, chocolate ice cream |
| 13120760 | Ice cream cone, chocolate covered or dipped, chocolate ice cream |
| 13120750 | Ice cream cone with nuts, chocolate ice cream |
| 53544250 | Granola bar, coated with non-chocolate coating |
| 13120780 | Ice cream cone, chocolate covered, with nuts, chocolate ice cream |
| 58148600 | Pasta tetrazzini, dry mix, prepared with water |
| 53109270 | Cake, cupcake, chocolate, with or without icing, fruit filling or cream filling, lowfat, cholesterol |
| 13140450 | Light ice cream, cone, NFS (formerly ice milk) |
| 51320040 | Roll, wheat or cracked wheat, made from home recipe or purchased at bakery |
| 51220030 | Roll, whole wheat, 100%, made from home recipe or purchased at bakery |
| 51220040 | Roll, whole wheat, 100%, made from home recipe or purchased at bakery, toasted |

**Mixtures containing dry milks**
(Adjusted for dry milk content of 1.01 to 5.0%)
[Astaxanthin] = 0.000017 to 0.00009%

| | |
|---|---|
| 53243050 | Cookie, vanilla sandwich, reduced fat |
| 54305500 | Crispbread, wheat or rye, extra crispy |
| 51301040 | Bread, wheat or cracked wheat, made from home recipe or purchased at bakery |
| 51301050 | Bread, wheat or cracked wheat, made from home recipe or purchased at bakery, toasted |
| 53105500 | Cake, chocolate, with icing, diet |
| 53220010 | Cookie, fruit-filled bar, fat free |
| 91304070 | Topping, peanut butter, thick, fudge type |
| 75649100 | Vegetable soup, cream of, made from dry mix, low sodium, prepared with water |
| 53220040 | Cookie, fig bar, fat free |
| 58310310 | Pancakes and sausage (frozen meal) |

| | |
|---|---|
| 58310110 | Frozen breakfast, NFS (frozen meal) |
| 58310210 | Sausage and french toast (frozen meal) |
| 13120810 | Ice cream soda, chocolate |
| 51301540 | Bread, French or Vienna, whole wheat, other than 100% or NS as to 100%, made from home recipe or purchase |
| 51301550 | Bread, French or Vienna, whole wheat, other than 100% or NS as to 100%, made from home recipe or purchase |
| 52304040 | Muffin, bran with fruit, lowfat |
| 53123500 | Cake, shortcake, with whipped topping and fruit, diet |
| 53207050 | Cookie, chocolate, with chocolate filling or coating, fat free |
| 53102300 | Cake, applesauce, diet, without icing |
| 13110000 | Ice cream, NFS |
| 13130100 | Light ice cream, NFS (formerly ice milk) |
| 53521300 | Doughnut, wheat, chocolate covered |
| 58127220 | Croissant sandwich, filled with chicken, broccoli, and cheese sauce |
| 27540180 | Chicken patty sandwich or biscuit |
| 53108100 | Cake, cupcake, chocolate, without icing or filling |
| 28110640 | Meatballs, Swedish, in sauce, with noodles (frozen meal) |

**Mixtures containing dry milks**
(Adjusted for dry milk content of 0.07 to 1.0%)
[Astaxanthin] = 0.0000011 to 0.000017%

| | |
|---|---|
| 53544200 | Granola bar, chocolate-coated |
| 54403050 | Popcorn, flavored |
| 58145113 | Macaroni or noodles with cheese, canned |
| 53104300 | Cake, carrot, diet |
| 26133150 | Porgy, battered, fried |
| 26107150 | Catfish, battered, fried |
| 26145150 | Smelt, battered, fried |
| 53544300 | Granola bar, high fiber, coated with non-chocolate yogurt coating |
| 28141640 | Chicken kiev with rice-vegetable mixture (frozen meal) |
| 58127210 | Croissant sandwich, filled with ham and cheese |
| 53108000 | Cake, cupcake, chocolate, NS as to icing |
| 27112010 | Salisbury steak with gravy (mixture) |
| 41502000 | Beans and franks, frozen dinner |
| 26100150 | Fish, NS as to type, battered, fried |
| 26100250 | Fish stick, patty, or fillet, NS as to type, battered, fried |
| 26109150 | Cod, battered, fried |
| 26115150 | Flounder, battered, fried |
| 26117150 | Haddock, battered, fried |
| 26125150 | Ocean perch, battered, fried |
| 26127150 | Perch, battered, fried |
| 26129150 | Pike, battered, fried |
| 26131150 | Pompano, battered, fried |
| 26137150 | Salmon, battered, fried |
| 26151150 | Trout, battered, fried |
| 26157150 | Whiting, battered, fried |
| 26304150 | Conch, battered, fried |
| 26311150 | Lobster, battered, fried |
| 75412010 | Eggplant, batter-dipped, fried |
| 58132713 | Pasta with tomato sauce and frankfurters or hot dogs, canned |
| 28160310 | Meat loaf in tomato sauce with potatoes, vegetable (frozen meal) |
| 58163400 | Flavored rice and pasta mixture, reduced sodium |
| 58163380 | Flavored rice and pasta mixture |
| 58132113 | Pasta with tomato sauce and cheese, canned |

| | |
|---|---|
| 75412060 | Eggplant parmesan casserole, regular |
| 63401070 | Fruit, chocolate covered |
| 24301210 | Duck, battered, fried |
| 75440170 | Vegetable sticks, breaded (including corn, carrots, and green beans) |
| 53104520 | Cheesecake, diet |
| 53104570 | Cheesecake, diet, with fruit |
| 27212050 | Beef and macaroni with cheese sauce (mixture) |

**Non-reconstituted dry milks**
(Adjusted for not being reconstituted, 24g of powder to 240mL of water)
[Astaxanthin] = 0.017%

| | |
|---|---|
| 11810000 | Milk, dry, not reconstituted, NS as to whole, lowfat, or nonfat |
| 11811000 | Milk, dry, whole, not reconstituted |
| 11812000 | Milk, dry, lowfat, not reconstituted |
| 11813000 | Milk, dry, nonfat, not reconstituted |

**Flavored Milk and Milk Drinks**
[Astaxanthin] = 0.0002%

| | |
|---|---|
| 11511000 | Milk, chocolate, NFS |
| 11511100 | Milk, chocolate, whole milk-based |
| 11511200 | Milk, chocolate, reduced fat milk-based (formerly "lowfat") |
| 11511300 | Milk, chocolate, skim milk-based |
| 11511400 | Milk, chocolate, lowfat milk-based |
| 11512000 | Cocoa, hot chocolate, not from dry mix, made with whole milk |
| 11512500 | Spanish-style hot chocolate drink, Puerto Rican style, made with evaporated milk |
| 11513000 | Cocoa and sugar mixture, milk added, NS as to type of milk |
| 11513100 | Cocoa and sugar mixture, whole milk added |
| 11513150 | Cocoa and sugar mixture, reduced fat milk added |
| 11513200 | Cocoa and sugar mixture, lowfat milk added |
| 11513300 | Cocoa and sugar mixture, skim milk added |
| 11514100 | Cocoa, sugar, and dry milk mixture, water added |
| 11514300 | Cocoa with nonfat dry milk and low calorie sweetener, mixture, water added |
| 11514500 | Cocoa, whey, and low calorie sweetener, mixture, fortified, water added |
| 11515100 | Cocoa and sugar mixture fortified with vitamins and minerals, milk added, NS as to type of milk, Puerto Rican |
| 11515400 | Cocoa with nonfat dry milk and low calorie sweetener, high calcium, water added |
| 11516000 | Cocoa, whey, and low-calorie sweetener mixture, lowfat milk added |
| 11518000 | Milk beverage with nonfat dry milk and low calorie sweetener, water added, chocolate |
| 11518050 | Milk beverage with nonfat dry milk and low calorie sweetener, water added, flavors other than chocolate |
| 11518100 | Milk beverage with nonfat dry milk and low calorie sweetener, high calcium, water added, chocolate |
| 11519000 | Milk beverage, made with whole milk, flavors other than chocolate |
| 11519050 | Milk, flavors other than chocolate, whole milk-based |
| 11520000 | Milk, malted, unfortified, NS as to flavor, made with milk |
| 11521000 | Milk, malted, unfortified, chocolate, made with milk |
| 11521010 | Milk, malted, unfortified, chocolate, made with skim milk |
| 11522000 | Milk, malted, unfortified, natural flavor, made with milk |
| 11525000 | Milk, malted, fortified, natural flavor, made with milk |
| 11526000 | Milk, malted, fortified, chocolate, made with milk |
| 11527000 | Milk, malted, fortified, NS as to flavor, made with milk |
| 11531000 | Eggnog, made with whole milk |

| | |
|---|---|
| 11531500 | Eggnog, made with 2% reduced fat milk (formerly eggnog, made with "2% lowfat" milk) |
| 11541000 | Milk shake, NS as to flavor or type |
| 11541400 | Milk shake with malt |
| 11541500 | Milk shake, made with skim milk, chocolate |
| 11541510 | Milk shake, made with skim milk, flavors other than chocolate |
| 11542000 | Carry-out milk shake, NS as to flavor |
| 11542100 | Carry-out milk shake, chocolate |
| 11542200 | Carry-out milk shake, flavors other than chocolate |
| 11551050 | Milk fruit drink |
| 11553000 | Fruit smoothie drink, made with fruit or fruit juice and dairy products |
| 11553100 | Fruit smoothie drink, NFS |
| 11560000 | Chocolate-flavored drink, whey- and milk-based |
| 11560020 | Flavored milk drink, whey- and milk-based, flavors other than chocolate |
| 11561010 | Cafe con leche prepared with sugar |

**Mixtures containing flavored milk and milk drinks**
(Adjusted for flavored milk or milk drink content of 42.6%)
[Astaxanthin] = 0.0002%

| | |
|---|---|
| 11552200 | Milk-based fruit drink |

**Non-reconstituted flavored milk and milk drinks**
(Adjusted for not being reconstituted, 28g of powder to 240mL of water)
[Astaxanthin] = 0.0017%

| | |
|---|---|
| 11830100 | Cocoa (or chocolate) with dry milk and sugar, dry mix, not reconstituted |
| 11830110 | Cocoa powder with nonfat dry milk and low calorie sweetener, dry mix, not reconstituted |
| 11830120 | Cocoa, whey, and low calorie sweetener, fortified, dry mix, not reconstituted |
| 11830140 | Chocolate, instant, dry mix, fortified with vitamins and minerals, not reconstituted, Puerto Rican style |
| 11830150 | Cocoa powder, not reconstituted (no dry milk) |
| 11830160 | Cocoa (or chocolate) flavored beverage powder with sugar, dry mix, not reconstituted |
| 11830170 | Cocoa, whey, and low-calorie sweetener mixture, not reconstituted |
| 11830200 | Milk, malted, dry mix, unfortified, not reconstituted, flavors other than chocolate |
| 11830210 | Milk, malted, dry mix, fortified, not reconstituted, flavors other than chocolate |
| 11830250 | Milk, malted, dry mix, unfortified, not reconstituted, chocolate |
| 11830260 | Milk, malted, dry mix, fortified, not reconstituted, chocolate |
| 11830400 | Milk beverage, powder, dry mix, not reconstituted, flavors other than chocolate |
| 11830450 | Milk beverage with sugar, dry milk, and egg white powder, dry mix, not reconstituted |
| 11830500 | Milk beverage, powder, with nonfat dry milk and low calorie sweetener, dry mix, not reconstituted |
| 11830550 | Milk beverage, powder, with nonfat dry milk and low calorie sweetener, dry mix, not reconstituted |
| 92291300 | Postum, dry powder |

**Milk-Based Meal Replacements, Ready-to-Drink**
[Astaxanthin] = 0.0002%

| | |
|---|---|
| 11611000 | Instant breakfast, fluid, canned |
| 11612000 | Instant breakfast, powder, milk added |
| 11613000 | Instant breakfast, powder, sweetened with low calorie sweetener, milk added |
| 11621000 | Diet beverage, liquid, canned |

| | |
|---|---|
| 11622000 | Diet beverage, powder, milk added |
| 11622010 | Diet beverage, powder, reconstituted with skim milk |
| 11623000 | Meal supplement or replacement, commercially prepared, ready-to-drink |
| 11641000 | Meal supplement or replacement, milk-based, high protein, liquid |

**Non-reconstituted milk based meal replacements**
(Adjusted for not being reconstituted, 15g of powder to 240mL of water)
[Astaxanthin] = 0.0032%

| | |
|---|---|
| 11835000 | Meal replacement or nutritional supplement, Cambridge diet formula, powdered, nonfat milk solids bas |
| 11835100 | Meal replacement, Amway's Nutrilite brand Positrim Drink Mix, powdered nonfat dry milk-based, dry, n |
| 11830800 | Instant breakfast, powder, not reconstituted |
| 11830810 | Instant breakfast, powder, sweetened with low calorie sweetener, not reconstituted |
| 11830850 | High calorie milk beverage, powder, not reconstituted |
| 11830900 | Protein supplement, milk-based, powdered, not reconstituted |
| 11830940 | Meal replacement, high protein, milk based, fruit juice mixable formula, powdered, not reconstituted |
| 11830950 | Nutrient supplement, milk-based, powdered, not reconstituted |
| 11830960 | Protein supplement, milk-based, sodium controlled, powdered, not reconstituted |
| 11830970 | Meal replacement, protein type, milk-based, powdered, not reconstituted |
| 11830980 | Protein supplement, milk-based, powdered, not reconstituted |
| 11830990 | Nutrient supplement, milk-based, powdered, not reconstituted |
| 11831500 | Nutrient supplement, milk-based, high protein, powdered, not reconstituted |
| 11832000 | Meal replacement, protein type, milk- and soy-based, powdered, not reconstituted |
| 11832500 | Meal replacement, protein type, milk-based, with sugar and artificial sweetener, not reconstituted |

**Yogurt**
[Astaxanthin] = 0.0002%

| | |
|---|---|
| 11410000 | Yogurt, NS as to type of milk or flavor |
| 11411010 | Yogurt, plain, NS as to type of milk |
| 11411100 | Yogurt, plain, whole milk |
| 11411200 | Yogurt, plain, lowfat milk |
| 11411300 | Yogurt, plain, nonfat milk |
| 11420000 | Yogurt, vanilla, lemon, or coffee flavor, NS as to type of milk |
| 11422000 | Yogurt, vanilla, lemon, maple, or coffee flavor, lowfat milk |
| 11424000 | Yogurt, vanilla, lemon, maple, or coffee flavor, nonfat milk, sweetened with low calorie sweetener |
| 11425000 | Yogurt, chocolate, NS as to type of milk |
| 11427000 | Yogurt, chocolate, nonfat milk |
| 11430000 | Yogurt, fruit variety, NS as to type of milk |
| 11432000 | Yogurt, fruit variety, lowfat milk |
| 11432500 | Yogurt, fruit variety, lowfat milk, sweetened with low-calorie sweetener |
| 11433000 | Yogurt, fruit variety, nonfat milk |
| 41420380 | Tofu yogurt |

**Mixtures containing yogurts**
(Adjusted for yogurt content of 75.0 to 98.0%)
[Astaxanthin] = 0.00015 to 0.000196%

| | |
|---|---|
| 11421000 | Yogurt, vanilla, lemon, or coffee flavor, whole milk |
| 11426000 | Yogurt, chocolate, whole milk |
| 11431000 | Yogurt, fruit variety, whole milk |
| 11444000 | Yogurt, fruit and nuts, NS as to type of milk |
| 11445000 | Yogurt, fruit and nuts, lowfat milk |
| 11423000 | Yogurt, vanilla, lemon, maple, or coffee flavor, nonfat milk |
| 83115000 | Yogurt dressing |
| 11433500 | Yogurt, fruit variety, nonfat milk, sweetened with low-calorie sweetener |

**Mixtures containing yogurt**
(Adjusted for yogurt content of 5.1 to 60.0%)
[Astaxanthin] = 0.00001 to 0.00012%

| | |
|---|---|
| 81103041 | Margarine-like spread, made with yogurt, stick, salted |
| 81104011 | Margarine-like spread, reduced calorie, about 40% fat, made with yogurt, tub, salted |
| 83107100 | Mayonnaise, made with yogurt |
| 53366000 | Pie, yogurt, frozen |
| 53441210 | Basbousa (semolina dessert dish) |
| 27516010 | Gyro sandwich (pita bread, beef, lamb, onion, condiments), with tomato and spread |
| 58124500 | Pastry, filled with potatoes and peas, fried |
| 53104580 | Cheesecake -type dessert, made with yogurt, with fruit |

**Processed Fruits and Fruit Juices**

**Fruit Drinks and Ades**
[Astaxanthin] = 0.0002%

| | |
|---|---|
| 92510110 | Apple drink |
| 92510120 | Apple-cherry drink |
| 92510150 | Apple juice drink |
| 92510170 | Apple-cranberry-grape juice drink |
| 92510200 | Apple-orange-pineapple juice drink |
| 92510220 | Apricot-pineapple juice drink |
| 92510310 | Banana-orange drink |
| 92510410 | Black cherry drink |
| 92510610 | Fruit drink |
| 92510630 | Fruit juice drink, NFS |
| 92510650 | Tamarind drink, Puerto Rican (Refresco de tamarindo) |
| 92510810 | Grapeade and grape drink |
| 92510820 | Grape juice drink |
| 92510910 | Grapefruit juice drink |
| 92510950 | Guava juice drink |
| 92511010 | Lemonade |
| 92511020 | Lemon-limeade |
| 92511110 | Limeade |
| 92511190 | Orange juice drink |
| 92511200 | Orange-mango juice drink |
| 92511220 | Orange drink |
| 92511230 | Orange-apricot juice drink |
| 92511240 | Orange-lemon drink |

| | |
|---|---|
| 92511250 | Citrus fruit juice drink |
| 92511260 | Orange-cranberry juice drink |
| 92511270 | Orange-peach juice drink |
| 92511280 | Orange-grape-banana juice drink |
| 92511290 | Papaya juice drink |
| 92511310 | Pineapple-grapefruit juice drink |
| 92511340 | Pineapple-orange juice drink |
| 92511350 | Orange-raspberry juice drink |
| 92511400 | Raspberry-flavored drink |
| 92511510 | Strawberry-flavored drink |
| 92520410 | Fruit drink, low calorie |
| 92520810 | Grape drink, low calorie |
| 92520910 | Lemonade, low calorie |
| 92530110 | Apple drink with vitamin C added |
| 92530210 | Black cherry drink with vitamin C added |
| 92530310 | Cherry drink with vitamin C added |
| 92530410 | Citrus drink with vitamin C added |
| 92530510 | Cranberry juice drink with vitamin C added |
| 92530520 | Cranberry-apple juice drink with vitamin C added |
| 92530610 | Fruit punch, fruit drink, or fruitade, with vitamin C added |
| 92530710 | Grape drink with vitamin C added |
| 92530810 | Grapefruit juice drink with vitamin C added |
| 92530840 | Guava juice drink with vitamin C added |
| 92530910 | Lemonade with vitamin C added |
| 92531010 | Orange drink and orangeade with vitamin C added |
| 92531020 | Orange breakfast drink, made from frozen concentrate |
| 92531030 | Orange breakfast drink |
| 92531110 | Pineapple-grapefruit juice drink with vitamin C added |
| 92531120 | Pineapple-orange juice drink with vitamin C added |
| 92531150 | Pineapple-orange-grapefruit juice drink with vitamin C added |
| 92531210 | Strawberry-flavored drink with vitamin C added |
| 92541010 | Fruit-flavored drink, made from sweetened powdered mix (fortified with vitamin C) |
| 92541020 | Lemonade-flavored drink, made from powdered mix, with sugar and vitamin C added |
| 92541040 | Lemonade-flavored drink, made from powdered mix, low calorie, with vitamin C added |
| 92541100 | Apple cider-flavored drink, made from powdered mix, with sugar and vitamin C added |
| 92541120 | Apple cider-flavored drink, made from powdered mix, low calorie, with vitamin C added |
| 92542000 | Fruit-flavored drink, made from powdered mix, mainly sugar, with high vitamin C added |
| 92544000 | Fruit-flavored drink, made from unsweetened powdered mix (fortified with vitamin C), with sugar added |
| 92550050 | Apple-white grape juice drink, low calorie, with vitamin C added |
| 92550110 | Cranberry juice drink, low calorie, with vitamin C added |
| 92550210 | Cranberry-apple juice drink, low calorie, with vitamin C added |
| 92550300 | Grapefruit juice drink, low calorie, with vitamin C added |
| 92550610 | Fruit-flavored drinks, punches, ades, low calorie, with vitamin C added |
| 92551600 | Citrus juice drink, low calorie |
| 92551700 | Juice drink, low calorie |
| 92552000 | Fruit-flavored drink, made from powdered mix with high vitamin C added, low calorie |
| 92552050 | Orange breakfast drink, low calorie |
| 92552100 | Orange-cranberry juice drink, low calorie, with vitamin C added |

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| | |
|---|---|
| 92582000 | Fruit-flavored drink, low calorie, calcium fortified |
| 92582050 | Fruit-flavored drink, vitamin and mineral fortified |
| 92582100 | Citrus juice drink, calcium fortified |
| 92582110 | Orange breakfast drink, calcium fortified |
| 92731000 | Fruit-flavored drink, non-carbonated, made from powdered mix, with sugar |
| 92741000 | Fruit-flavored drink, non-carbonated, made from low calorie powdered mix |
| 92530950 | Vegetable and fruit juice drink, with vitamin C added |

**Non-reconstituted fruit drinks and ades**
(Adjusted for not being reconstituted, 1 cup of juice mix to 3 cups of water)
[Astaxanthin] = 0.0008%

| | |
|---|---|
| 92511000 | Lemonade, frozen concentrate, not reconstituted |

**Non-reconstituted fruit drinks and ades**
(Adjusted for not being reconstituted, 15g of powder to 240mL of water)
[Astaxanthin] = 0.0032%

| | |
|---|---|
| 92900100 | Tang, dry concentrate |
| 92900110 | Fruit-flavored concentrate, dry powder, with sugar and vitamin C added |
| 92900200 | Fruit-flavored beverage, dry concentrate, low calorie, not reconstituted |

**Processed Vegetables and Vegetable Juices**

**Vegetable Juices**
[Astaxanthin] = 0.0002%

| | |
|---|---|
| 73105010 | Carrot juice |
| 74301100 | Tomato juice |
| 74301150 | Tomato juice, low sodium |
| 74302000 | Tomato juice cocktail |
| 74303000 | Tomato and vegetable juice, mostly tomato |
| 74303100 | Tomato and vegetable juice, mostly tomato, low sodium |
| 75132000 | Mixed vegetable juice (vegetables other than tomato) |
| 75132100 | Celery juice |
| 75200700 | Aloe vera juice |

**Mixtures containing vegetable juices**
(Adjusted for vegetable juice content of 23.7 to 77.3%)
[Astaxanthin] = 0.00005 to 0.0002%

| | |
|---|---|
| 74501010 | Tomato aspic |
| 74304000 | Tomato juice with clam or beef juice |
| 93301030 | Bloody Mary |
| 28340660 | Chicken or turkey vegetable soup, home recipe |
| 27350310 | Seafood stew with potatoes and vegetables (including carrots, broccoli, and/or dark-green leafy), tomato |
| 27350030 | Seafood stew with potatoes and vegetables (excluding carrots, broccoli, and dark-green leafy), tomato |
| 28350120 | Crab soup, tomato-base |

**Mixtures containing vegetable juices**
(Adjusted for vegetable juice content of 5.4 to 16.9%)
[Astaxanthin] = 0.00001 to 0.00003%

| | |
|---|---|
| 27313220 | Beef, noodles, and vegetables (excluding carrots, broccoli, and dark-green leafy), tomato-based sauce |
| 27336150 | Venison/deer stew with potatoes and vegetables (excluding carrots, broccoli, and dark-green leafy), |
| 27430510 | Veal goulash with vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), tomato |
| 27336100 | Venison/deer stew with potatoes and vegetables (including carrots, broccoli, and/or dark-green leafy |
| 27111100 | Beef goulash |
| 27130050 | Lamb or mutton goulash |
| 27430500 | Veal goulash with vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), tomato |
| 27315270 | Stuffed grape leaves with beef and rice |
| 27315210 | Beef, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), tomato-based sauce |
| 27313210 | Beef, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), tomato-based sauce |
| 27315220 | Beef, rice, and vegetables (excluding carrots, broccoli, and/or dark-green leafy), tomato-based sauce |
| 27211100 | Beef stew with potatoes, tomato-based sauce (mixture) |
| 27211150 | Beef goulash with potatoes |
| 27360010 | Goulash, NFS |
| 27330170 | Stuffed grape leaves with lamb and rice |
| 27212150 | Beef goulash with noodles |

**Soft Candy**

**Chocolate Confections and Other Soft Candy**
[Astaxanthin] = 0.0013%

| | |
|---|---|
| 91700500 | M & M's Almond Chocolate Candies |
| 91701010 | Almonds, chocolate covered |
| 91701020 | Almonds, sugar-coated |
| 91701030 | Almonds, yogurt-covered |
| 91702010 | Butterscotch morsels |
| 91703010 | Caramel, chocolate-flavored roll |
| 91703020 | Caramel, flavor other than chocolate |
| 91703030 | Caramel, with nuts |
| 91703040 | Caramel, chocolate covered |
| 91703050 | Caramel with nuts and cereal, chocolate covered |
| 91703060 | Caramel with nuts, chocolate covered |
| 91703070 | Rolo |
| 91703080 | Caramel, all flavors, sugar free |
| 91703150 | Toblerone, milk chocolate with honey and almond nougat |
| 91703200 | TWIX Cookie Bars |
| 91703250 | TWIX Chocolate Fudge Cookie Bars |
| 91703300 | TWIX Peanut Butter Cookie Bars |
| 91703400 | Whatchamacallit |
| 91703500 | Nuts, carob-coated |
| 91703600 | Espresso coffee beans, chocolate-covered |
| 91705010 | Chocolate, milk, plain |
| 91705020 | Chocolate, milk, with cereal |

| | |
|---|---|
| 91705030 | Kit Kat |
| 91705040 | Chocolate, milk, with nuts, not almond or peanuts |
| 91705050 | Chocolate, milk, with fruit and nuts |
| 91705060 | Chocolate, milk, with almonds |
| 91705070 | Chocolate, milk, with peanuts |
| 91705090 | Chocolate with fondant and caramel |
| 91705200 | Chocolate, semi-sweet morsel |
| 91705300 | Chocolate, sweet or dark |
| 91705400 | Chocolate, white |
| 91705410 | Chocolate, white, with almonds |
| 91705420 | Chocolate, white, with cereal |
| 91705500 | Mexican chocolate (tablet) |
| 91706000 | Coconut candy, chocolate covered |
| 91706100 | Coconut candy, no chocolate covering |
| 91706400 | Coconut candy, Puerto Rican style |
| 91707000 | Fondant |
| 91707010 | Fondant, chocolate covered |
| 91708000 | Fruit peel, candied |
| 91708010 | Date candy |
| 91708020 | Soft fruit confections |
| 91708030 | Fruit leather |
| 91708040 | Fun Fruits Creme Supremes |
| 91708070 | Tamarind candy |
| 91708100 | Fruit snack candy, with added vitamin C |
| 91709000 | Gumdrops, chocolate covered |
| 91713010 | Fudge, chocolate, chocolate-coated |
| 91713020 | Fudge, chocolate, chocolate-coated, with nuts |
| 91713030 | Fudge, chocolate |
| 91713040 | Fudge, chocolate, with nuts |
| 91713050 | Fudge, peanut butter |
| 91713060 | Fudge, peanut butter, with nuts |
| 91713070 | Fudge, vanilla |
| 91713080 | Fudge, vanilla, with nuts |
| 91713090 | Fudge, divinity |
| 91713100 | Fudge, brown sugar (penuche) |
| 91715000 | Fudge, caramel and nut, chocolate-coated |
| 91715100 | SNICKERS Bar |
| 91715200 | Baby Ruth |
| 91715300 | 100 GRAND Bar |
| 91716010 | Halvah, plain |
| 91716110 | Halvah, chocolate covered |
| 91718100 | Butterfinger |
| 91718200 | Chocolate-flavored sprinkles |
| 91718300 | Ladoo, round ball, Asian-Indian dessert |
| 91721000 | Licorice |
| 91723000 | Marshmallow |
| 91723010 | Marshmallow, chocolate covered |
| 91723020 | Marshmallow, candy-coated |
| 91723050 | Marshmallow, coconut-coated |
| 91726000 | Nougat, plain |
| 91726110 | Nougat, with caramel, chocolate covered |
| 91726130 | MILKY WAY Bar |
| 91726140 | MILKY WAY DARK Bar |
| 91726150 | MARS Bar |
| 91726410 | Nougat, chocolate covered |
| 91726420 | 3 MUSKETEER Bar |

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| | |
|---|---|
| 91727010 | Nuts, chocolate covered, not almonds or peanuts |
| 91728000 | Nut roll, fudge or nougat, caramel and nuts |
| 91728500 | Sugared pecans (sugar and egg white coating) |
| 91731000 | Peanuts, chocolate covered |
| 91731010 | M & M's Peanut Chocolate Candies |
| 91731060 | M & M's Peanut Butter Chocolate Candies |
| 91731100 | Peanuts, sugar-coated |
| 91731150 | Peanuts, yogurt covered |
| 91732000 | Peanut bar |
| 91732100 | Planters Peanut Bar |
| 91733000 | Peanut brittle |
| 91733200 | Peanut Bar, chocolate covered |
| 91734000 | Peanut butter, chocolate covered |
| 91734100 | Reese's Peanut Butter Cup |
| 91734200 | Reese's Pieces |
| 91734300 | Reese Sticks |
| 91734400 | Reese's Fast Break |
| 91734500 | Peanut butter morsels |
| 91735000 | Pralines |
| 91736000 | Pineapple candy, Puerto Rican style |
| 91739010 | Raisins, chocolate covered |
| 91739510 | Raisins, carob covered |
| 91739600 | Raisins, yogurt covered |
| 91742010 | Sesame Crunch (Sahadi) |
| 91745010 | Gumdrops |
| 91745100 | Skittles |
| 91746010 | Sugar-coated chocolate discs |
| 91746100 | M & M's Plain Chocolate Candies |
| 91746120 | Sixlets |
| 91746150 | Easter egg, candy coated chocolate |
| 91750000 | Taffy |
| 91750110 | Food Stick, fortified |
| 91760000 | Toffee, plain |
| 91760100 | Toffee, chocolate covered |
| 91760200 | Toffee, chocolate-coated, with nuts |
| 91760500 | Truffles |
| 91760700 | Wax candy, liquid filled |
| 91770010 | Dietetic or low calorie gumdrops |
| 91770030 | Dietetic or low calorie candy, chocolate covered |

SUBMISSION END

AM

**Mcmahon, Carrie**

**From:** csacra@easconsultinggroup.com
**Sent:** Wednesday, August 12, 2009 12:27 PM
**To:** Mcmahon, Carrie
**Subject:** FW: GRAS Notice No GRN 294
**Attachments:** Literature short list for GRN 294.pdf; 1-A-Nishikawa et al 1997 English.pdf; 1-B-Nishikawa et al 1997 Japanese.pdf; 2-Takahashi et al 90-day English.pdf

Dear Carrie,

Here is the first of 3 emails with the attached articles you requested for our GRN 294. All article files are listed numerically with author name that correspond with your request list. Please note that in some of the translated articles (paper 1 and 6) Tables or Figures are not included and a copy of the Japanese article with the Tables and Figures is also included. Additionally, please note that for article 5 only the Japanese version is included. Our write up in the GRAS monograph was based on the English version and from secondary citations for this article. We have requested the English translation which we expect to get soon. I will email that to you as soon as possible and will then send you one full set of all your requested articles for the administrative copy.

Best regards,
Cathryn

Cathryn W. Sacra
EAS Consulting Group, LLC
1940 Duke Street
Suite 200
Alexandria, VA 22314
703-684-4438
703-684-4428-fax
www.easconsultinggroup.com

**From:** csacra@easconsultinggroup.com [mailto:csacra@easconsultinggroup.com]
**Sent:** Friday, August 07, 2009 10:23 AM
**To:** 'Madhu Soni'
**Subject:** FW: GRAS Notice No GRN 294

Madhu,

I am forwarding this request from Carrie McMahon at FDA for English translations of the attached papers.

Thanks,
Cathryn

**From:** Mcmahon, Carrie [mailto:Carrie.McMahon@fda.hhs.gov]
**Sent:** Thursday, August 06, 2009 1:51 PM
**To:** csacra@easconsultinggroup.com
**Subject:** RE: GRAS Notice No GRN 294

000150

Cathryn -

Per our telephone conversation today, I have attached a PDF file listing the literature, cited in your GRAS notice (GRN 000294; *Haematococcus pluvialis* extract containing astaxanthin esters), for which we are requesting copies in English. This list is a "short list" of the articles which our toxicology reviewer feels are pivotal to our ability to evaluate Fuji Chemical's GRAS determination.

Regards,

Carrie

Carrie H. McMahon, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review

tel: (301) 436-1202
email: Carrie.McMahon@fda.hhs.gov

**From:** csacra@easconsultinggroup.com [mailto:csacra@easconsultinggroup.com]
**Sent:** Thursday, August 06, 2009 12:29 PM
**To:** Mcmahon, Carrie
**Cc:** esteele@easconsultinggroup.com
**Subject:** GRAS Notice No GRN 294

Dear Carrie,

I am responding to your request for some documents in connection with GRN 294. If you would send me an email of what you require, I will get the documentation as soon as possible.

Best regards,
Cathryn

Cathryn W. Sacra
EAS Consulting Group, LLC
1940 Duke Street
Suite 200
Alexandria, VA 22314
703-684-4438
703-684-4428--Fax

AM

## Mcmahon, Carrie

**From:** csacra@easconsultinggroup.com
**Sent:** Wednesday, August 12, 2009 12:30 PM
**To:** Mcmahon, Carrie
**Subject:** FW: GRAS Notice No GRN 294 2 0f 3
**Attachments:** 6-A-Nitta et al 2005 English.pdf; 4-Ono et al 1999 English-Japanese.pdf; 5-Onodera et al 1997 Japanese.pdf

Email 2 of 3

**From:** csacra@easconsultinggroup.com [mailto:csacra@easconsultinggroup.com]
**Sent:** Friday, August 07, 2009 10:23 AM
**To:** 'Madhu Soni'
**Subject:** FW: GRAS Notice No GRN 294

Madhu,

I am forwarding this request from Carrie McMahon at FDA for English translations of the attached papers.

Thanks,
Cathryn

**From:** Mcmahon, Carrie [mailto:Carrie.McMahon@fda.hhs.gov]
**Sent:** Thursday, August 06, 2009 1:51 PM
**To:** csacra@easconsultinggroup.com
**Subject:** RE: GRAS Notice No GRN 294

Cathryn -

Per our telephone conversation today, I have attached a PDF file listing the literature, cited in your GRAS notice (GRN 000294; *Haematococcus pluvialis* extract containing astaxanthin esters), for which we are requesting copies in English. This list is a "short list" of the articles which our toxicology reviewer feels are pivotal to our ability to evaluate Fuji Chemical's GRAS determination.

Regards,

Carrie

Carrie H. McMahon, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review

tel: (301) 436-1202
email: Carrie.McMahon@fda.hhs.gov

**From:** csacra@easconsultinggroup.com [mailto:csacra@easconsultinggroup.com]
**Sent:** Thursday, August 06, 2009 12:29 PM

AM

## Mcmahon, Carrie

**From:** csacra@easconsultinggroup.com
**Sent:** Wednesday, August 12, 2009 12:30 PM
**To:** Mcmahon, Carrie
**Subject:** FW: GRAS Notice No GRN 294 3 0f 3
**Attachments:** 7-MalmstenLingell-2008.pdf; 8-Tsukhara et al 2005.pdf; 9-Nagaki et al 2005.pdf; 3-Ohgami et al 2005.pdf

Email 3 of 3

**From:** csacra@easconsultinggroup.com [mailto:csacra@easconsultinggroup.com]
**Sent:** Friday, August 07, 2009 10:23 AM
**To:** 'Madhu Soni'
**Subject:** FW: GRAS Notice No GRN 294

Madhu,

I am forwarding this request from Carrie McMahon at FDA for English translations of the attached papers.

Thanks,
Cathryn

**From:** Mcmahon, Carrie [mailto:Carrie.McMahon@fda.hhs.gov]
**Sent:** Thursday, August 06, 2009 1:51 PM
**To:** csacra@easconsultinggroup.com
**Subject:** RE: GRAS Notice No GRN 294

Cathryn -

Per our telephone conversation today, I have attached a PDF file listing the literature, cited in your GRAS notice (GRN 000294; *Haematococcus pluvialis* extract containing astaxanthin esters), for which we are requesting copies in English. This list is a "short list" of the articles which our toxicology reviewer feels are pivotal to our ability to evaluate Fuji Chemical's GRAS determination.

Regards,

Carrie

Carrie H. McMahon, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review

tel: (301) 436-1202
email: Carrie.McMahon@fda.hhs.gov

**From:** csacra@easconsultinggroup.com [mailto:csacra@easconsultinggroup.com]
**Sent:** Thursday, August 06, 2009 12:29 PM
**To:** Mcmahon, Carrie

000153

AM

EAS CONSULTING GROUP

*Specializing in FDA Regulatory Matters*

RECEIVED
AUG 24 2009
Division of Biotechnology and GRAS Notice Review

August 19th, 2009

Carrie H. McMahon, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review
HFS-255
5100 Paint Branch Road
College Park, MD 20740-3835

Dear Dr. McMahon:

**RE: GRN 294**

Enclosed, please find English translations of the requested articles for GRN 294, as well as a new page 40, which contains the signatures of the expert panel. Note that the dates of the signatures post-date the submission of GRN 294. When EAS Consulting Group initially submitted the GRAS notification in June, 2009, page 40 of the original submission contained a fully signed page 40. However, FDA requested some alterations to the submission. EAS submitted a revised version on July 7, 2009 which unfortunately did not contain a page signed by the expert panel. I have circulated a new page 40 to be included in GRN 294 for your records and apologize for any inconvenience that this has caused you.

If you have any further questions, please feel free to contact me at 703-684-4438.

Sincerely,

Cathryn W. Sacra

Encls

000154

EAS Consulting Group, LLC
1940 Duke Street, Suite 200, Alexandria, Virginia 22314
(877) 327-9808 Toll Free • (703) 684-4408 Local • (703) 684-4428 Fax

## 4. CONCLUSION

Based on a critical evaluation of the publicly available data summarized herein, the Expert Panel members whose signatures appear below, have individually and collectively concluded that astaxanthin esters derived from *Haematococcus pluvialis*, meeting the specifications cited herein, and when used as a nutrient in Baked Goods, Beverages, Cereals, Chewing Gum, Coffee and Tea, Dairy Product Analogs, Frozen Dairy Desserts and Mixes, Hard Candy, Milk Products, Processed Fruits and Fruit Juices, Processed Vegetables and Vegetable Juices, and Soft Candy at use levels of 0.5 mg astaxanthin *per* serving (reference amounts customarily consumed, 21 CFR 101.12) and when not otherwise precluded by Standards of Identity are safe.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that astaxanthin esters, when used as described, are GRAS based on scientific procedures.

**Signatures**

| | |
|---|---|
| Stanley M. Tarka, Jr. Ph.D. | 12 August 2009<br>Date |
| John A. Thomas, Ph.D., F.A.T.S., D.A.T.S. | 12 August 2009<br>Date |
| Madhusudan G. Soni, Ph.D., F.A.C.N. | 17 August 2009<br>Date |

## 4. CONCLUSION

Based on a critical evaluation of the publicly available data summarized herein, the Expert Panel members whose signatures appear below, have individually and collectively concluded that astaxanthin esters derived from *Haematococcus pluvialis*, meeting the specifications cited herein, and when used as a nutrient in Baked Goods, Beverages, Cereals, Chewing Gum, Coffee and Tea, Dairy Product Analogs, Frozen Dairy Desserts and Mixes, Hard Candy, Milk Products, Processed Fruits and Fruit Juices, Processed Vegetables and Vegetable Juices, and Soft Candy at use levels of 0.5 mg astaxanthin *per* serving (reference amounts customarily consumed, 21 CFR 101.12) and when not otherwise precluded by Standards of Identity are safe.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that astaxanthin esters, when used as described, are GRAS based on scientific procedures.

**Signatures**

| | |
|---|---|
| Stanley M. Tarka, Jr. Ph.D. | 12 August 2009<br>Date |
| John A. Thomas, Ph.D., F.A.T.S., D.A.T.S. | 13 August 2009<br>Date |
| Madhusudan G. Soni, Ph.D., F.A.C.N. | 17 August 2009<br>Date |

000156

Pages 000157 - 000163 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000164 - 000170 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000171 - 000193 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000194 - 000208 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000209 - 000219 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000220 - 000227 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000228 - 000235 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000236 - 000244 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000245 - 000252 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000253 - 000273 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000274 - 000276 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000277 - 000292 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000293 - 000301 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

AM

## Mcmahon, Carrie

**From:** csacra@easconsultinggroup.com
**Sent:** Tuesday, October 20, 2009 1:15 PM
**To:** Mcmahon, Carrie
**Subject:** RE: GRN 294
**Attachments:** RetinalBloodFlow_ExpertLetter-1.pdf; E. Steele Ltr.pdf; Fox CV_August 2009.pdf

Dear Carrie,

Attached is an electronic copy of the response to the questions from your review team on GRN 294. Please note that Appendix II is a separate document from Dr. Donald A. Fox. A copy of Dr. Fox's CV is also attached. I will be sending a hard copy by mail for your files.

Please contact me at 703-684-4438 if you have any questions.

Best regards,
Cathryn

Cathryn W. Sacra
EAS Consulting Group, LLC
1940 Duke Street
Suite 200
Alexandria, VA 22314
703-684-4438
703-684-4428-fax
www.easconsultinggroup.com

**From:** Mcmahon, Carrie [mailto:Carrie.McMahon@fda.hhs.gov]
**Sent:** Thursday, September 17, 2009 10:24 AM
**To:** csacra@easconsultinggroup.com
**Subject:** RE: GRN 294

Cathryn -

I have received the input of the review team on the questions we have regarding your notice (GRN 000294; H. pluvialis extract containing astaxanthin esters). Please see the attached PDF file.

If you have any questions, please do not hesitate to contact me.

Regards,

Carrie H. McMahon, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review

tel: (301) 436-1202
email: Carrie.McMahon@fda.hhs.gov

**From:** csacra@easconsultinggroup.com [mailto:csacra@easconsultinggroup.com]
**Sent:** Wednesday, September 16, 2009 1:07 PM
**To:** Mcmahon, Carrie
**Subject:** RE: GRN 294

Thanks. I just wanted to be sure I didn't miss anything

**From:** Mcmahon, Carrie [mailto:Carrie.McMahon@fda.hhs.gov]
**Sent:** Wednesday, September 16, 2009 12:51 PM
**To:** csacra@easconsultinggroup.com
**Subject:** RE: GRN 294

Cathryn -

I have not yet sent the questions. As noted in my voicemail, I'm working with the reviewers to make sure the text clearly and accurately relays their questions. I will send these along once I have the reviewers' revisions.

You may call me if you have any questions.

Regards,

Carrie

Carrie H. McMahon, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review

tel: (301) 436-1202
email: Carrie.McMahon@fda.hhs.gov

**From:** csacra@easconsultinggroup.com [mailto:csacra@easconsultinggroup.com]
**Sent:** Wednesday, September 16, 2009 12:44 PM
**To:** Mcmahon, Carrie
**Subject:** GRN 294

Dear Carrie,

I got your phone message last week concerning questions that the scientific panel had on GRN 294. You mentioned that you would send an email with those questions. I wanted to check to be sure that you had not sent the questions. I haven't received them, but I have had some problems with my email in the past few days and wanted to ensure that the questions had not gone astray.

Thanks so much,
Cathryn

Cathryn W. Sacra
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Alexandria, VA 22314
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Typo

~~November 19,~~ 2009

Carrie H. McMahon, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review
5100 Paint Branch Road
College Park, MD 20740-3835

Dear Dr. McMahon,

**RE: *Haematococus pluvialis* extract containing astaxanthin esters (GRN 294)**

This responds to your email of September 17, 2009 regarding questions from your review team on our GRAS notice (GRN 000294; *Haematococus pluvialis* extract containing astaxanthin esters). We are providing a point-by-point response to your questions along with some discussion and an opinion letter from an expert researcher in the areas of retinal cell biology and vision sciences.

**Identity, Method of Manufacture and Composition**

1. FDA Query: Please revise Section II (pages 3-4) of the notice to state clearly that the subject of the notice is a mixture of astaxanthin esters and to present relevant information, if available, for astaxanthin esters. The current version of the notice may create the impression that the notice is about pure astaxanthin.

Response: As suggested, we have revised this section of the notice. Please see below Appendix I.

2. FDA Query: You describe two extraction methods in your manufacturing process: supercritical $CO_2$ extraction and acetone extraction. You provide a specification for residual acetone of not more than 5 ppm but do not identify the step in the manufacturing process that removes residual acetone. Please describe how residual acetone is removed to meet your specification.

Response: The acetone is removed from the extract by evaporation under reduced pressure and molecular distillation under vacuum. Initially, evaporation under reduced pressure is employed at ~40°C for gross removal of acetone. This step is followed by molecular distillation under vacuum at >100°C to further remove the residual acetone.

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AM

## Mcmahon, Carrie

**From:** csacra@easconsultinggroup.com
**Sent:** Wednesday, October 21, 2009 1:34 PM
**To:** Mcmahon, Carrie
**Subject:** RE: GRN 294
**Attachments:** Fox CV_August 2009.pdf; RetinalBloodFlow_ExpertLetter-1.pdf; GRN 294 - Final Response.pdf

Dear Carrie,

My apologies. That was a clerical error on my part. I have attached a new signed letter dated October 21, and had attached Appendix II and Dr. Fox's CV again.

Thanks,
Cathryn

---

**From:** Mcmahon, Carrie [mailto:Carrie.McMahon@fda.hhs.gov]
**Sent:** Tuesday, October 20, 2009 2:05 PM
**To:** csacra@easconsultinggroup.com
**Subject:** RE: GRN 294

Cathryn -

Could you check your copy of Ed's letter? The email attachment I have gives a date of **November** 19.

Carrie McMahon, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review

tel: (301) 436-1202
email: Carrie.McMahon@fda.hhs.gov

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---

**From:** csacra@easconsultinggroup.com [mailto:csacra@easconsultinggroup.com]
**Sent:** Tuesday, October 20, 2009 1:15 PM
**To:** Mcmahon, Carrie
**Subject:** RE: GRN 294

Dear Carrie,

Attached is an electronic copy of the response to the questions from your review team on GRN 294. Please note that Appendix II is a separate document from Dr. Donald A. Fox. A copy of Dr. Fox's CV is also attached. I will be sending a hard copy by mail for your files.

Please contact me at 703-684-4438 if you have any questions.

Attachment:
GRN 294 - Final Response.pdf

*Specializing in FDA Regulatory Matters*

October 21, 2009

Carrie H. McMahon, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review
5100 Paint Brand Road
College Park, MD 20740-3835

Dear Dr. McMahon,

**RE: *Haematococus pluvialis* extract containing astaxanthin esters (GRN 294)**

This responds to your email of September 17, 2009 regarding questions from your review team on our GRAS notice (GRN 000294; *Haematococus pluvialis* extract containing astaxanthin esters). We are providing a point-by-point response to your questions along with some discussion and an opinion letter from an expert researcher in the areas of retinal cell biology and vision sciences.

**Identity, Method of Manufacture and Composition**

1. FDA Query: Please revise Section II (pages 3-4) of the notice to state clearly that the subject of the notice is a mixture of astaxanthin esters and to present relevant information, if available, for astaxanthin esters. The current version of the notice may create the impression that the notice is about pure astaxanthin.

Response: As suggested, we have revised this section of the notice. Please see below Appendix I.

2. FDA Query: You describe two extraction methods in your manufacturing process: supercritical $CO_2$ extraction and acetone extraction. You provide a specification for residual acetone of not more than 5 ppm but do not identify the step in the manufacturing process that removes residual acetone. Please describe how residual acetone is removed to meet your specification.

Response: The acetone is removed from the extract by evaporation under reduced pressure and molecular distillation under vacuum. Initially, evaporation under reduced pressure is employed

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3. FDA Query: We have noticed that the analytical results given in Appendix I account for only about 92% of the composition of your product, while the results in Table 5 (p. 14), which were obtained in a different laboratory add up to 99%. Please explain this discrepancy in the results of the analyses.

Response: The analysis reported in Appendix I was performed at Japan Food Research Lab (JFRL), a Ministry of Agriculture, Forestry and Fisheries (MAFF) Japan authorized laboratory. The JFRL report accounts for about 92% of the composition of the extract. As we understand, within JFRR, different independent labs performed the analysis of different parameters at different time from the same batch/lot. As analysis of the different constituents was performed at different laboratories, and the total was less than 100%, Fuji contracted for a complete new analysis with an independent laboratory for the Lot 1 (Appendix I of the GRAS notice) sample that was analyzed by JFRL. The results of this analysis accounted for up to 99% of the constituents (Table 5, p. 14). Thus, the discrepancy for the compositional analysis was related to the analysis by JFRL.

**Safety Assessment (toxicological data)**

4. FDA Query: On page 11 of your GRAS notice (Section 1. *Introduction* to the GRAS panel statement), you state that "[a] comprehensive search of the scientific literature for safety and toxicity information on astaxanthin was conducted through May 2009 and made available to the Expert Panel. The Expert Panel independently and critically evaluated materials submitted by Fuji Chemical Industry and other information deemed appropriate or necessary." FDA notes that a significant number of the references cited in the GRAS panel statement were not originally published in English. As your GRAS determination relies in part on the conclusions of your GRAS panel, we request that you confirm if the members of your GRAS panel have --- in the course of their evaluation of the literature --- reviewed the English translations of the literature referenced in the GRAS Panel Statement.

Response: The primary toxicologist, who is also a member of the expert panel, reviewed all the articles, including translated articles from Japanese publications. This toxicologist prepared the GRAS monograph and submitted it to other panel members. The other panelists had an access to all the articles along with the translated articles. We have confirmed with other panelists that they have reviewed the English translated articles.

5. FDA Query: The majority of information in your GRAS notice is based upon the safety of astaxanthin from *H. pluvialis* for consumption by healthy individuals; further, several of your references report the effects of astaxanthin on parameters pertaining to the eye in normal individuals. Several studies specifically excluded individuals with diabetes.

- FDA Query: We note that the findings of Nagaki, Y. et al. (Journal of Clinical Therapeutic Medicine. 2005; 21:537-542) showed an increase in retinal capillary blood flow in human subjects following dietary supplementation with 6 mg/day of astaxanthin. In your GRAS notice, you estimate a daily intake of astaxanthin from the intended uses of the extract to be 5.4mg/day at the 90th percentile all-user intakes.
- FDA Query: We further note that there is published evidence showing a possible relationship between retinal capillary blood flow and risk of diabetic retinopathy progression. Pregnant diabetic women, who are at increased risk for the progression of diabetic retinopathy, have increased retinal capillary blood flow during pregnancy and after delivery in comparison to pregnant, non-diabetic women. (Investigative Ophthalmology and Visual Science. 2003;44:1486-1491, 2003).
- FDA Query: Finally, we note that individuals with diabetes make up a significant proportion of the U.S. population and that "between 40 and 45 percent of Americans diagnosed with diabetes have some stage of diabetic retinopathy." However, all people with diabetes— both type 1 and type 2—are at risk for diabetic retinopathy. (www.nei.nih.gov/health/diabetic/retinopathy.asp).

FDA Query: Given (1) evidence supporting the effect of astaxanthin on retinal capillary blood flow and (2) that diabetic individuals are predisposed to abnormal and fragile blood vessels of the retina -- and are at risk for diabetic retinopathy -- please confirm whether you (and/or your GRAS panel) considered the potential effects of astaxanthin on individuals with diabetes/diabetic retinopathy from the consumption of foods containing astaxanthin esters from *H. pluvialis*. If you did consider this issue, please discuss your considerations and state your conclusions. If you did not, please explain why consideration of the effects of astaxanthin on individuals with diabetes/diabetic retinopathy is not relevant to your GRAS determination.

Response:

As regards exclusion of diabetic subjects, although the majority of the human clinical studies were conducted in healthy subjects, the exclusion was not related to any concern for safety of diabetic subjects, but to avoid or minimize the effects of a disease condition, if any, as a confounding factor in the evaluation. However, in a recently published human study (Hashimoto et al., 2009), which has been translated into English and made available to our panel and vision science expert, the effect of astaxanthin consumption on superoxide-scavenging activity in aqueous humor of diabetic subjects was investigated. In this study, 35 patients (diabetic and non-

diabetic) that were scheduled for cataract surgery on both eyes participated. Surgery on one eye was performed before astaxanthin consumption, while surgery on the other eye was performed after astaxanthin consumption (for 2 weeks, 6 mg/day) and superoxide scavenging activity was measured by superoxide dismutase activity, reduced glutathione and ascorbic acid. Superoxide-scavenging activity was significantly improved (increased) in the intact cataract eye following astaxanthin consumption in the diabetic group.

As regards the effects of astaxanthin on retinal capillary blood flow and its implications to diabetic individuals, particularly pregnant diabetic women, the available evidence indicate that the dynamics of the processes involved during astaxanthin-induced retinal blood flow and in diabetic retinopathy are different and that astaxanthin is unlikely to cause adverse effects in subjects with diabetes/diabetic retinopathy. A series of studies related to the mechanism of astaxanthin action support this notion. Available evidence suggests that astaxanthin improves eye function; possesses anti-diabetic, anti-hypertensive and anti-inflammatory potentials. Additionally, the technology, science and interpretation of the data involved with retinal blood flow measurements and outcome values is still a developing area.

A. Improved eye function: The available evidence from multiple (over 10) double-blind placebo-controlled human clinical studies suggest that astaxanthin improves eye function without any adverse effects. Given this clinical evidence, the increase in retinal capillary blood flow following astaxanthin ingestion may be considered beneficial rather than adverse. Astaxanthin-induced improvement in blood flow might enhance delivery of glucose, oxygen and other nutrients and may also facilitate removal of metabolic waste products. This may enhance functions of the retinal pigment epithelium, photoreceptor cells, retinal ganglion cells and optic nerve. As regards the blood flow in diabetic pregnant women, it is not clear that hyperdynamic retinal capillary circulation contributes to the progression of diabetic retinopathy as opposed to the increased capillary blood flow in these women reflecting an altered vascular system. The pregnancy facilitates the progression of retinopathy by independent pathways, including clinical variables associated with diabetes and hypertension. Available evidence from animal and human studies suggests that astaxanthin possesses anti-diabetic and anti-hypertensive effects. At present, there is no consensus among the scientific community on the proper methodology or data analysis technique to measure and analyze ocular blood flow. The available evidence indicates that the technique used in the measurement of the blood flow need to be optimized to provide more reliable and valid conclusions. In this regard, the opinion obtained from an independent expert in vision sciences is also enclosed (Appendix II).

   In an experimental study, Izumi-Nagai et al. (2008) reported that astaxanthin treatment suppressed laser photocoagulation induced choroidal neovascularization (CNV)

development in C57BL/6J. A significant inhibition of macrophage infiltration into CNV and of the *in vivo* and *in vitro* expression of inflammation-related molecules, including VEGF, IL-6, ICAM-1, MCP-1, VEGFR-1, and VEGFR-2 was noted following astaxanthin treatment. Astaxanthin suppressed the activation of the NF-kB pathway that leads to inflammation. Available evidence suggests that VEGF plays an important role in retinal vascularization during development and in pathological neovascularization in ischemic retinopathies. Damage to retinal blood vessels resulting in closure of retinal capillaries and retinal ischemia occurs in several disease processes, including diabetic retinopathy. As astaxanthin protects from inflammation, it is unlikely to cause any damage during diabetic retinopathy.

B. Anti-diabetic potential: Available evidence from *in vitro* and *in vivo* experimental studies suggests that astaxanthin may actually prevent the progression of diabetic nephropathy or pathogenesis associated with increased oxidative stress. Naito et al. (2002) reported that astaxanthin may prevent diabetic nephropathy. In this study, administration of astaxanthin to female db/db mice, a rodent model of type-2 diabetes, for 12 weeks resulted in lower levels of blood glucose, ameliorated relative mesangial area, and inhibited urinary albumin and 8-hydroxydeoxyguanosine excretion. The results suggest that astaxanthin ameliorated the progression or acceleration of diabetic nephropathy. In another similar study, Uchiyama et al. (2002) demonstrated that treatment with astaxanthin for over 12 weeks improved glycemic control with preservation of pancreatic-cell function in diabetic mouse model (db/db mice).

In a recent six-week study, Naito et al. (2006) examined gene expression profile of glomerular cells in the early phase of diabetic nephropathy in db/db mice and identified the mitochondrial oxidative phosphorylation pathway as the caronical pathway that was most significantly affected by diabetic nephropathy in mice. In this study, treatment with astaxanthin significantly decreased the expression of upregulated genes, including those associated with oxidative phosphorylation, oxidative stress, and the TGF-β collagen synthesis system. In an *in vitro* study, Manabe et al. (2007) reported that astaxanthin exposure to normal human mesangial cells (specialized cells around blood vessels in the kidneys), cultured in the medium containing normal (5 mM) or high (25 mM) concentrations of D-glucose, resulted in accumulation of astaxanthin in the mitochondria of the cells that possibly scavenged overproduced reactive oxygen species (ROS), reduced ROS-modified protein production and inhibited subsequent transcription factors activation and COX-2, MCP-1, and TGF 1 production. These molecular mechanistic changes indicate that astaxanthin might prevent the progression of diabetic nephropathy. These investigators also reported that astaxanthin suppressed high glucose induced activation of NFkB, which is known to be involved in ROS related diabetic complications.

C. Anti-inflammatory activity: Suzuki et al. (2006) investigated the effects of astaxanthin on endotoxin-induced uveitis (EIU) in rats by measuring the expression of inflammatory cytokines and chemokines. In this study, EIU was induced in male Lewis rats by footpad injection of lipopolysaccharide (LPS) and animals were treated intravenously with astaxanthin (1, 10, or 100 mg/kg). Aqueous humour was collected at 6, 12 and 24 hours and the number of infiltrating cells in the anterior chamber was counted and concentration of protein, nitric oxide (NO), tumor necrosis factor-α (TNF-α), prostaglandin E2 (PGE2) and NF-kB activation was assayed. Astaxanthin injections showed a significant decrease in the number of infiltrating cells in the anterior chamber of LPS injected animals. Additionally there was a significantly lower concentration of protein, NO, TNF-α and PGE2 in the aqueous humour of astaxanthin-treated animals injected with LPS. The number of activated NF-kB-positive cells was lower in iris-ciliary bodies treated with 10 or 100 mg/kg astaxanthin at 3 hours after LPS injection. These results suggest that astaxanthin reduced ocular inflammation.

Based on the results of *in vitro* and *in vivo* investigations, Nakajima et al. (2008) suggested that astaxanthin protects retinal cells against oxidative stress. In the *in vitro* study, retinal damage was induced by 24-hour hydrogen peroxide treatment or serum deprivation in cultured retinal ganglion cell line. Astaxanthin inhibited the neurotoxicity induced by hydrogen peroxide and serum deprivation and reduced the intracellular oxidation induced by various reactive oxygen species. Additionally, astaxanthin decreased the radical generation induced by serum deprivation. In the *in vivo* study, oral administration of astaxanthin (100 mg/kg, four times) to mice reduced the retinal damage (as assessed by lipid peroxidation and DNA damage) induced by N-methyl-D-aspartate injection.

Iwamoto et al. (2000) determined the *in vitro* and *in vivo* effects of astaxanthin on low-density lipoprotein (LDL) oxidation. The oxidation of LDL was measured in a reaction system consisting of increasing concentrations of astaxanthin, 2,2'-azobis(4-methoxy-2, 4-dimethylvaleronitrile) and LDL. Compared to control, astaxanthin significantly prolonged the oxidation lag time in a dose –dependent relationship. In the *in vivo* study 24 volunteers consumed astaxanthin at doses of 1.8, 3.6, 14.4 and 21.6 mg/day for 14 days. Fasting venous blood samples were taken at baseline and at the end of study. LDL lag time was longer (5.0, 26.2, 42.3 and 30.7% respectively) compared with baseline after consuming astaxanthin at doses of 1.8, 3.6,14.4 and 21.6 mg for 14 days. The results of this suggest that astaxanthin inhibits LDL oxidation. Other studies have shown that astaxanthin did not exhibit pro-oxidant potential (Martin et al., 1999; Goto et al., 2001). Additionally, as discussed above, Hashimoto et al. (2009) reported significant improvement (increase) in superoxide-scavenging activity in human diabetic subjects following astaxanthin consumption.

Bursell et al. (1999) investigated the role of antioxidants in normalizing retinal blood flow and renal function in patients with <10 years of type 1 diabetes. In this 8-month randomized double-masked placebo-controlled crossover trial, 36 type 1 diabetic and 9 nondiabetic subjects received either 1800 IU vitamin E/day or placebo for 4 months and followed, after treatment crossover, for a further 4 months. Baseline retinal blood flow as measured by video fluorescein angiography in diabetic subjects was significantly lower compared with that of nondiabetic subjects. Following vitamin E supplementation, diabetic patient retinal blood flow was significantly increased and was comparable with that of nondiabetic subjects. Oral vitamin E treatment appears to be effective in normalizing retinal hemodynamic abnormalities and improving renal function in type 1 diabetic patients. The results of this study suggest that antioxidant supplementation may provide an additional benefit in reducing the risks for developing diabetic retinopathy or nephropathy. As an antioxidant, astaxanthin is about ten times more powerful compared to other carotenoids and Vitamin E (Miki et al., 1982; Terao, 1989).

D. Anti-hypertensive potential: In a series of studies in normotensive and hypertensive rats, Hussein et al. (2005a; 2005b; 2006; 2007) investigated the potential anti-hypertensive effects of astaxanthin. The results of these investigations suggest the antihypertensive effects (Hussein et al., 2005a; 2005b; 2006) and the mechanism of action (Hussein et al., 2005a) of astaxanthin in hypertensive rats. In these studies, Wistar Kyoto (WKY) rats or spontaneously hypertensive (SHR) rats were used. In the 2007 study, this group investigated the effects of astaxanthin on metabolic syndrome in SHR/NDmcr-cp (cp/cp), a spontaneously hypertensive rat model of metabolic syndrome (referred to as SHRcp). In this study, oral administration of astaxanthin (50 mg/kg body weight/day) for 22 weeks induced a significant reduction in arterial blood pressure in SHRcp, fasting blood glucose level, homeostasis index of insulin resistance, and improved insulin sensitivity. The results also showed an improved adiponectin level, a significant increase in high-density lipoprotein cholesterol, a significant decrease in plasma levels of triglycerides, and non-esterified fatty acids. The results of this study suggest that astaxanthin ameliorates insulin resistance by mechanisms involving the increase of glucose uptake, and by modulating the level of circulating lipid metabolites and adiponectin. The conclusion that astaxanthin lowered the blood glucose level of SHRcp in insulin tolerance tests is supported by a previous observation that astaxanthin protects pancreatic β-cells against glucose toxicity by preventing the progressive destruction of these cells in diabetic db/db mice (Uchiyama et al., 2002).

In one of the studies, Hussein et al. (2006) also analyzed the post-treatment effects of astaxanthin on the vascular tissues by examining the changes in the aorta and coronary arteries and arterioles. In this study oral administration of astaxanthin (5 mg/kg body weight/day for 7 weeks) to SHR rats showed a significant reduction in the elastin bands

in the rat aorta. Astaxanthin administration also significantly decreased the [wall:lumen] aerial ratio of the coronary arteries. The results of this study suggest that astaxanthin can modulate the oxidative condition and may improve vascular elastin and arterial wall thickness in hypertension. These observations indicate that astaxanthin is likely to protect the fragile blood vessels from rupture. Contrary to these observations, arterial hypertension and diabetic vascular complications are connected with an elevated degradation of elastic tissue.

E. Retinal blood flow: A letter from Dr. Donald A. Fox addressing this issue is enclosed (Appendix II).

**Summary and Conclusion**

This response provides additional information related to identity, manufacturing and composition as desired by FDA and discusses safety related concerns raised by FDA. Primarily, FDA's safety concern was related to the effects of astaxanthin on individuals with diabetic retinopathy. The clinical evidence that astaxanthin improves eye function and shows possess anti-diabetic, anti-hypertensive and anti-inflammatory potentials, suggest that the mechanisms involved in the increased retinal blood flow following ingestion of astaxanthin are different than noted in pregnant diabetic women. It appears that the pregnancy alters the progression of retinopathy by independent pathways, including clinical variables associated with diabetes and hypertension. Astaxanthin appears to improve eye function and prevent the progression of diabetic nephropathy and pathological conditions without any adverse effects. The totality of the available evidence and discussion presented above show that astaxanthin consumption is unlikely to cause adverse effects in subjects with diabetes/diabetic retinopathy and is considered safe at the intended use levels in the specified foods as described in our GRAS notice. This conclusion is further supported by the enclosed opinion letter from an independent expert in vision sciences familiar with retinal blood flow and diabetic retinopathy.

If you have any questions or need additional explanation, we will be happy to further discuss this.

Thank you for the opportunity to provide this explanation.

Best regards

Edward A. Steele
President

**References**

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**Appendix I**

## II Detailed Information about the Identity of the Notified Substance:

### A. Chemical Name

Mixture of astaxanthin esters derived from *H. pluvialis*. Astaxanthin is chemically known as (3S,3'S)-3,3'-dihydroxy-beta,beta-carotene-4,4'-dione.

### B. Trade Name:

The subject of this notification will be marketed as AstaREAL®. Fuji Chemical Industry Co., Ltd. manufactures different types of products (*H. pluvialis* extract) characterized by astaxanthin esters that are marketed under the trade name AstaREAL® as a food ingredient. Currently, these products include AstaREAL® (formerly known as AstraCarox®), AstaREAL® Oil 50F, and AstaREAL® L10. AstaREAL® (dry biomass) which contains free and esterified astaxanthin (total of 5% in the free form as measured after hydrolysis). AstaREAL® Oil 50F, which is marketed in Japan, also contains 5% astaxanthin in its free form, and is prepared by employing an acetone extraction process. AstaREAL® L10 which contains 10% astaxanthin in its free form, is marketed in the U.S., and is prepared by employing a supercritical $CO_2$ extraction process.

### C. Chemical Abstract Registry Number:

There is no CAS number for astaxanthin esters. The CAS number for astaxanthin is: 472-61-7.

### D. Chemical Formula:

As astaxanthin esters derived from *H. pluvialis* are formed with different fatty acids the chemical formula varies and depends on particular fatty acid. The empirical formula of the astaxanthin molecule in astaxanthin esters is $C_{40}H_{52}O_4$

### E. Structure:

The structure of astaxanthin ester depends on the fatty acid moiety. The structural diagram of the astaxanthin molecule is presented below (Figure 1).

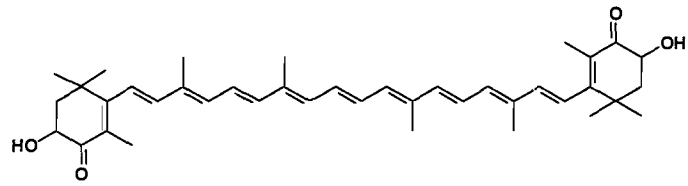


**Figure 1. Chemical structure of astaxanthin**

## F. Molecular Weight

Not applicable as the substance is a mixture. Molecular weight for astaxanthin is: 596.8 Daltons.

## G. Physical Characteristics

*H. pluvialis* extract characterized by astaxanthin esters is produced as dark red viscous oils.

## H. Typical Composition and Specifications

Typical compositional analysis and specifications of *H. pluvialis* extract (characterized by astaxanthin esters) manufactured by employing supercritical $CO_2$ extraction are presented in Tables 1 and 2. Compositional analysis of *H. pluvialis* extract characterized by astaxanthin esters manufactured by employing acetone extraction method is similar to that of a supercritical $CO_2$ extraction method except the acetone extracted product has higher values for tocopherols (6.1%) and phospholipids (0.2%). The slightly higher levels of tocopherol and phospholipids may be related to the polar nature of the extraction solvent acetone. The specification of astaxanthin esters prepared by both methods is essentially the same. For the acetone extraction method an additional specification for acetone (<5 ppm) is included.

**Table 1. Compositional analysis of *H. pluvialis* extract characterized by astaxanthin esters**

| | Typical value | Assay method |
|---|---|---|
| Acylglycerol | | |
| Triacylglycerol | 53.5 %,w/w | JFRL Method |
| Diacylglycerol | 17.7 %, w/w | |
| Monoacylglycerol | ND | |
| Subtotal | 71.5 %, w/w | |
| Free fatty acids | 0.5 %, w/w | JOCS 3.3.5; JP 2.02 |
| Carotenoids | | Reverse phase HPLC |
| Astaxanthin | 15.7 %, w/w | |

**Table 1. Compositional analysis of *H. pluvialis* extract characterized by astaxanthin esters**

| | Typical value | Assay method |
|---|---|---|
| Others* | 1.1 %, w/w | |
| Subtotal | 16.8 %, w/w | |
| Tocopherols | 2.6 %, w/w | Normal phase HPLC |
| Phospholipids | 0.042 %, w/w | JOCS 2.4.11 |
| Moisture | 0.27 | JP 2.48 |
| Total | 91.4 %**, w/w | |

JFRL = Japan Food Research Lab, JP = Japanese Pharmacopoeia, JOCS = Japan Oil Chemists' Society. *Include lutein, adonirubin, β-carotene, catnthaxanthin **As analysis for different constituents was performed at different laboratories, the total may be less than 100%

**Table 2. Specifications for *H. pluvialis* extract characterized by astaxanthin esters***

| | Specification | Typical value | Assay method |
|---|---|---|---|
| Description | Dark red viscous oil | Complies | Visual test |
| Astaxanthin | NLT 10% (As free form by HPLC) | 10.9% | JP 2.24 & JP 2.01 |
| Pheophorbide | Less than 20 mg/dL | Complies | Kanshoku No.99** |
| Heavy metals | Less than 10 ppm | Complies | NMKL 161 |
| Arsenic | Less than 2 ppm | Complies | NMKL 161 |
| Lead | Less than 1 ppm | Complies | NMKL 161 |
| Mercury | Less than 1 ppm | Complies | NMKL 170 |
| Cadmium | Less than 1 ppm | Complies | NMKL 161 |
| Microbiological assays | | | |
| Total plate count | Less than 1000 cfu/g | Complies | NMKL 86,4 2006 |
| Yeast and Mold | Less than 100 cfu/g | Complies | NMKL 98 |
| Coliform bacteria | Negative/g | Negative | NMKL 44, 6, 2004 |
| *Staphylococcus aureus* | Negative/g | Negative | NMKL 66 |
| *Salmonella* | Negative/g | Negative | NMKL 125,4 2005 |

*Astaxanthin esters prepared by employing acetone extraction method have an additional specification of acetone NMT 5 ppm cfu = colony forming units, JP = Japanese Pharmacopoeia, NMKL = Nordic Committee on Food Analysis, **Notice from Environmental Health Bureau-Ministry of Health and Welfare, Japan dated May 8th, 1981

A typical fatty acid profile of astaxanthin esters from the extract is summarized in Table 3. The fatty acid profile of astaxanthin esters from the extract prepared by employing the supercritical $CO_2$ extraction method or the acetone extraction method is essentially the same.

**Table 3. Fatty acid profile of astaxanthin esters**

| Fatty acids | Percentage* |
|---|---|
| C12:0 | 0.4 |
| C14:0 | 0.4 |
| C16:0 (palmitic acid) | 15.7 |
| C17:1 | 3 8 |
| C18:0 | 4.0 |
| C18:1 (oleic acid) | 30.7 |
| C18:2 (n-6) (linoleic acid) | 27.7 |
| C18:3 (n-6) | 1.2 |
| C18:3 (n-3) (linolenic acid) | 11.5 |
| C18:4 (n-3) | 1.2 |
| C20:0 | 0.7 |
| C20:1 | 0.1 |
| C20:2 (n-6) | 1.1 |
| C20:4 (n-6) | 1.0 |
| C20:5 (n-3) | 0.3 |
| C22:0 | 0.1 |
| Others | 0.1 |
| Total | 100 |

*Percent of total fatty acids

Appendix II

Letter from Dr. Donald A. Fox, Retinal Cell Biology and Vision Sciences Consultant to Expert Panel; Attached separately

Attachment:
RetinalBloodFlow-ExpertLetter-1.pdf

**Dr. Donald A. Fox**
(b) (6)

**Appendix II** October 15, 2009

Carrie H. McMahon, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review

Dear Dr. McMahon

My name is Donald A. Fox. I am a Professor of Vision Sciences, Biology and Biochemistry, and Pharmacology at the University of Houston. I have conducted research studies in the areas of retinal cell and molecular biology, anatomy, biochemistry, nutrition, pathology, pharmacology, physiology and toxicology as well as human disease for the past 30 years. In addition, I have taught courses in general and ocular pharmacology, eye diseases and pathology, nutrition and basic visual science for the past 30 years. I am the senior author on the chapter "Toxic responses of the ocular and visual system" in *Casarett & Doull's Toxicology: The Science of Poisons*, 7th Edition (Ed., Klaassen, C.D., McGraw-Hill Press: New York). My CV is attached.

I have been requested by the Expert Panel for the Astaxanthin GRAS to provide additional insight into factors that affect retinal blood flow and to provide a response to the FDA query on astaxanthin (AX). I am an independent consultant. Below, I will briefly summarize the current literature on blood flow in diabetic subjects and in pregnant diabetics subjects and provide a summary opinion on the risk potential of AX as a GRAS in diabetic and pregnant diabetic subjects.

Retinal blood flow is a common measure in patients with diabetic retinopathy, since it is thought to reflect the course and risk of retinal damage. However, these studies show decreased retinal blood flow, no change or increased retinal blood during pregnancy in diabetic patients. (Cunha-Vaz et al. 1978; Kawagishi et al. 1995; Bursell et al. 1996; Cuypers et al. 2000; Michelson et al. 2001; Schocket et al. 2005). The reasons



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for the diversity and discordance of findings are that retinal blood flow in diabetics depends upon several different important factors. One is the clinically assessed stage of diabetes: stage 0 - no diabetic retinopathy; stages 1 and 2 - non-proliferative with mild or moderate exudates; stage 3 - severe non-proliferative or pre-proliferative; stages 4 and 5 - proliferative (Aldington et al. 1995; Bursell et al. 1996; Yun et al. 2008). Generally, decreases in blood flow are found in stages 0, 1 and 2; no change or decreased blood flow is seen in stage 5; and increased blood flow is observed during stages 3 and 4 (Cunha-Vaz et al. 1978; Aldington et al. 1995; Cuypers et al. 2000; Yun et al. 2008). Moreover, blood flow differs in different regions of the retina (i.e., nasal, papillo-macular and perifoveal), and therefore measurements in multiple areas are needed to adequately determine statistically significant changes (Cuypers et al. 2000; Michelson et al. 2001). Additionally, the arterial partial pressure of oxygen and blood glucose level influence retinal blood flow rates (Sullivan et al. 1990; Bursell et al. 1996; Strenn et al. 1997). Moreover, even with the modern non-invasive Heidelberg Retinal Flowmeters and sophisticated software, the variation in trial-to-trial measurement is approximately 5% (Ludovico et al. 2003) and the intra-observer variability is 11-16% (Cuypers et al. 2000).

There are a limited number of retinal blood flow studies in diabetic women during pregnancy and the postpartum period (Schocket et al. 1999; Loukovaara et al. 2002; Loukovaara et al. 2003). The results are equivocal. Results from one laboratory that measured retinal blood flow from one retinal region showed a 6% increase from the first to third trimester, and either a 3% increase or 4% decrease from the third trimester to the third postpartum month relative to controls (Loukovaara et al. 2002; Loukovaara et al. 2003). The other study, that measured blood flow in two retinal regions, found an 11% decrease from the third trimester to the third to fourth postpartum month relative to controls (Schocket et al. 1999). Neither study found a progression of retinopathy, although both theorized for different reasons that such changes could occur. For most pregnant diabetic women, the progression of diabetic retinopathy occurs temporarily during pregnancy and postpartum and it has no long-term detrimental effects unless it progressed to late stage 4 or 5 (Temple et al. 2001; Kaaja and Loukovaara 2007; Sheth 2008).

Nagaki et al. (2005) conducted a four-week exposure study to 6 mg of AX and analyzed the safety and retinal blood flow in 18 placebo controls and 18 AX subjects: all healthy 40 year old subjects. There were no safety issues. They measured blood flow in the right and left eyes with a Heidelberg Retinal Flowmeter. After four weeks of AX ingestion, they reported a 9-10% increase in subjects that ingested AX, compared to a 3% increase in the placebo group.

In summary, I conducted a detailed review of the literature on retinal blood flow in diabetic subjects and in pregnant diabetic subjects as well as of the manuscript of Nagaki et al. (2005). A brief unbiased summary of the results of the blood flow studies and their equivocal outcomes with lack of progression to diabetic retinopathy was

presented. I conclude that AX as a GRAS will not produce an increased risk for diabetic retinopathy in diabetic or pregnant diabetic subjects.

Respectfully,

Donald A. Fox

Digitally signed by Donald A. Fox
DN: CN = Donald A. Fox, C = US, O = UHCO
Date: 2009.10.15 18:07:24 -05'00'

Donald A. Fox, PhD., Fellow of ATS, Fellow of ARVO
Professor of Vision Sciences, Biology and Biochemistry, and Pharmacology
Retinal Cell Biology and Vision Sciences Consultant to the Expert Panel

## REFERENCES


Aldington SJ, Kohner EM, Meuer S, Klein R, Sjølie AK. Methodology for retinal photography and assessment of diabetic retinopathy: the EURODIAB IDDM complications study. Diabetologia 38: 437-444, 1995.

Bursell SE, Clermont AC, Kinsley BT, Simonson DC, Aiello LM, Wolpert HA. Retinal blood flow changes in patients with insulin-dependent diabetes mellitus and no diabetic retinopathy. Invest Ophthalmol Vis Sci 37: 886-897, 1996.

Cunha-Vaz JG, Fonseca JR, de Abreu JR, Lima JJ. Studies on retinal blood flow. II. Diabetic retinopathy. Arch Ophthalmol 96: 809-811, 1978.

Cuypers MH, Kasanardjo JS, Polak BC. Retinal blood flow changes in diabetic retinopathy measured with the Heidelberg scanning laser Doppler flowmeter. Graefes Arch Clin Exp Ophthalmol 238: 935-941, 2000.

Fox DA, Boyes WK. Toxic responses of the ocular and visual system, In *Casarett & Doull's Essentials of Toxicology.* 7th Edition. Eds., Klaassen, C.D. and Watkins, J.B., McGraw-Hill Press: New York, pp. 665-697, 2007.

Inada H, Isshiki G, Morii H. Impaired retinal artery blood flow in IDDM patients before clinical manifestations of diabetic retinopathy.Diabetes Care 18: 1544-1549, 1995.

Kaaja R, Loukovaara S. Progression of retinopathy in type 1 diabetic women during pregnancy. Curr Diabetes Rev 3: 85-93, 2007.

Kawagishi T, Nishizawa Y, Emoto M, Konishi T, Maekawa K, Hagiwara S, Okuno Y, Loukovaara S, Harju M, Kaaja R, Immonen I. Retinal capillary blood flow in diabetic and nondiabetic women during pregnancy and postpartum period. Invest Ophthalmol Vis Sci 44: 1486-1491, 2003.

Loukovaara S, Kaaja R, Immonen I. Macular capillary blood flow velocity by blue-field entoptoscopy in diabetic and healthy women during pregnancy and the postpartum period. Graefes Arch Clin Exp Ophthalmol 240: 977-982, 2002.

Ludovico J, Bernardes R, Pires I, Figueira J, Lobo C, Cunha-Vaz J. Alterations of retinal capillary blood flow in preclinical retinopathy in subjects with type 2 diabetes. Graefes Arch Clin Exp Ophthalmol 241: 181-186, 2003.

Michelson G, Welzenbach J, Pal I, Harazny J. Functional imaging of the retinal microvasculature by scanning laser Doppler flowmetry. Int Ophthalmol 23: 327-335, 2001.

Nagaki Y, Mihara M, Takahashi J, Kitamura A, Horita Y, Sugiura Y, Tsukahara H. The effect of astaxanthin on retinal capillary blood flow in normal volunteers. J Clin Therapeut Med 21: 537-542, 2005.

Schocket LS, Grunwald JE, Tsang AF, DuPont J. The effect of pregnancy on retinal hemodynamics in diabetic versus nondiabetic mothers. Am J Ophthalmol 128: 477-484, 1999.

Sheth BP. Does pregnancy accelerate the rate of progression of diabetic retinopathy?: an update. Curr Diab Rep 8: 270-273, 2008.

Strenn K, Menapace R, Rainer G, Findl O, Wolzt M, Schmetterer L. Reproducibility and sensitivity of scanning laser Doppler flowmetry during graded changes in PO2. Br J Ophthalmol 81: 360-364, 1997.

Sullivan PM, Davies GE, Caldwell G, Morris AC, Kohner EM. Retinal blood flow during hyperglycemia. A laser Doppler velocimetry study. Invest Ophthalmol Vis Sci 31: 2041-2045, 1990.

Temple RC, Aldridge VA, Sampson MJ, Greenwood RH, Heyburn PJ, Glenn A. Impact of pregnancy on the progression of diabetic retinopathy in Type 1 diabetes. Diabet Med 18: 573-577, 2001.

Yun YL, Acharya UR, Venkatesh YV, Chee C, Min LC, Ng EYK. Identification of different stages of diabetic retinopathy using retinal optical images. Information Sciences 178: 106-121, 2008.

Attachment:
Fox CV_August 2009.pdf



# CURRICULUM VITAE
# of
# Donald A. Fox, Ph.D.

## CONTACT INFORMATION

University of Houston
College of Optometry
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Houston, TX 77204-2020
U.S.A.

713.743.1964 Office & Voice mail
713.743.1998 or 713.743.1871 Laboratory
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dafox@uh.edu
http://www.bchs.uh.edu/People/Fox_D/Fox_D.html

## EDUCATION

| | |
|---|---|
| Postdoctoral | NIEHS Individual Postdoctoral Fellowship: Neurotoxicology and Neurophysiology, University of California: Davis, CA, 1977-1979. |
| Ph.D., 1977 | NIEHS Predoctoral Fellowship Toxicology and Neurobiology, University of Cincinnati Medical Center, Department of Environmental Health, Kettering Laboratory, Cincinnati, OH, 1972-1977. |
| B.S., 1970 | Chemistry, Miami University, Oxford, OH, 1966-1970. |

## PROFESSIONAL EXPERIENCE

| | |
|---|---|
| 2006 | Keck Faculty Affiliate, Keck Center for Bioscience Training, The Gulf Coast Consortia, Houston, TX. |
| 2001 | Professor, Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX. |
| 1998 | Professor, Department of Biology and Biochemistry, University of Houston, Houston, TX. |
| 1993 | Professor, College of Optometry, University of Houston, Houston, TX. |
| 1993 | Professor, Department of Biochemical and Biophysical Sciences, University of Houston, Houston, TX. |
| 1990-1993 | Associate Professor, Department of Biochemical and Biophysical Sciences, University of Houston, Houston, TX. |

| | |
|---|---|
| 1986 | Visiting Scholar from May-November at Northwestern University, Evanston, IL, Department of Biomedical Engineering, Laboratory of Dr. Christina Enroth-Cugell. |
| 1986-Present | Director of Pharmacology Program, College of Optometry, University of Houston, Houston, TX. |
| 1985-1993 | Associate Professor, College of Optometry, University of Houston, Houston, TX. |
| 1982-1985 | Assistant Professor, College of Optometry, University of Houston, Houston, TX. |
| 1979-1982 | Assistant Professor of Toxicology and Pharmacology, Division of Toxicology, Department of Pharmacology, The University of Texas Medical School at Houston, Houston, TX. |
| 1977-1979 | Postdoctoral Fellow, Departments of Animal Physiology and Environmental Toxicology, University of California, Davis, CA. |
| 1976-1977 | Research Pharmacologist, U. S. Environmental Protection Agency, Health Effects Research Laboratory, Toxicology Division, Cincinnati, OH. |

## HONORS AND AWARDS

Faculty Opponent for Ph.D. Thesis Defense at the Karolinska Institute, Institute of Environmental Medicine, Stockholm, Sweden (Elisabetta Dare: Defendant; Sandra Ceccatelli: Advisor), 2002.

Faculty Opponent for Ph.D. Thesis Defense at the Karolinska Institute, Institute of Environmental Medicine, Stockholm, Sweden (Anna Rossi: Defendant; Pierluigi Nicotera: Advisor), 1996.

Biographical Profile in Marquis Who's Who In Medicine and Healthcare, First Edition: 1997 and American Men and Women in Science, 1997

City of Houston Proclamation for Outstanding Service to the University, 1993

University of Houston Research Excellence Award, Associate Professor, 1991-1992

Frank R. Blood Award from Society of Toxicology, February, 1992 (Best Research Paper of 1991 published in the official SOT journal: Toxicology and Applied Pharmacology)

Prominent Teaching Professor at University of Houston, 1990-1991 (UH Campus-wide Graduate Student Teaching Award)

NIEHS Postdoctoral Fellowship

NIEHS Predoctoral Fellowship

B.S., Honors in Chemistry

## EDITORIAL POSITIONS

Associate Editor of *Environmental Health Perspectives* (2008-Present)

Associate Editor of *Toxicology and Applied Pharmacology* (2002-2005; 2005-Present)

Member, Board of Publication of *Mitochondrion* (2004-Present)

Associate Editor of *Journal of Ocular Pharmacology and Toxicology* (2004-Present)

Editorial Board of *Cutaneous and Ocular Pharmacology* (2005-Present)

Editorial Board of *Molecular Vision* (1995-Present)

Editorial Board of *The Open Ophthalmology Journal.* (2007-Present)

Editorial Board of *Toxicology and Applied Pharmacology* (1991-1996; 2001-2002)

Editorial Board of *NeuroToxicology* (1980-1986; 1996-2002)

Associate Editor of *NeuroToxicology* (1986-1996)

Editorial Board of *Toxicology and Ecotoxicology* (1993-1998)

Editorial Board of *Neurotoxicology and Teratology* (1979-1987)

## NIH ADVISORY PANELS

National Toxicology Program (NTP) Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) (2005-2009).

NIH/FDA Advisory Panel on Ocular Toxicology for ICCVAM & NICETAM (2004-2005)

## NIH AND OTHER STUDY/GRANT REVIEW SECTIONS

NIEHS NAL Study Section for Toxicology (2003-2005)
NIEHS Special Study Section for Toxicology (2001, 2003)
NIEHS Study Section for Minority Grants (1999; 2002)
NIEHS Study Section for Superfund Grants (1994; 1997)
Expert Reviewer for American Heart Association Study Section (2000; 2001)
US. Civilian Research and Development Foundation (CRDF) Grant Review (2001)
Expert Reviewer for Vision Sciences C Study Section: NEI (1994; 1996; 1997)

## SERVICE ON NATIONAL AND INTERNATIONAL ADVISORY COMMITTEES

Nominated by Deputy Directors of NTP and NCTR/FDA for The Science Advisory Committee on Alternative Toxicological Methods (SACATM). (2005).

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). 2004-2005.

National Alliance for Eye and Vision Research Advocacy Committee: 2000-2002.

Association for Research in Vision and Ophthalmology (ARVO) EdNet Committee: 1999-2000.

Scientific Organizing Committee and Continuing Education Committee for 8th International Congress of Toxicology held in Paris, France in July, 1998: Committees from 1996-1998.

National Academy of Sciences, National Research Council, Committee on Vision, Neurotoxicology of Vision Committee, 1989-1990.

FDA Committee on Optical Radiation and Visual Health, Section on Drug Photoxicity and Visual Health, 1986.

National Academy of Sciences, National Research Council, Safe Drinking Water Committee, Neurotoxicology Sub-committee, 1984-1985.

National Academy of Sciences, National Research Council, Committee on Vision, 1982-1985.

National Committee on Air Quality Control, Washington, D.C., 1979-1980.

United States Environmental Protection Agency, Committee on Behavioral Test Methods for Neurotoxic Substances, 1978-1979.

Technical Committee, Society of Toxicology, 1991-1993: Chair, 1992-1993.

Continuing Education Committee, Society of Toxicology, 1987-1991.

Program Committee, Society of Toxicology, 1984-1987.

Task Force on Communication, Society of Toxicology, 1983-1984.

**PROFESSIONAL SOCIETY MEMBERSHIPS**

American Association for the Advancement of Science (AAAS)
American Chemical Society (ACS)
American Society of Cell Biology (ASCB)
Association for Research in Vision and Ophthalmology (ARVO)
Neurobehavioral Toxicology Society
New York Academy of Sciences
Sigma Xi
Society for Neuroscience (SfN)
Society of Toxicology (SOT)

## ELECTED NATIONAL POSITIONS

President, International Neurotoxicology Association, 2009-2011.

President-Elect, International Neurotoxicology Association, 2007-2008.

Councilor, International Neurotoxicology Association, 2004-2008.

Association for Research in Vision and Ophthalmology (ARVO) Retinal Cell Biology Program Committee: Member, 1998-2002; Chair, 2000-2001.

President, Neurotoxicology Specialty Section, Society of Toxicology, 1988-1989.

Vice-President, Neurotoxicology Specialty Section, Society of Toxicology, 1987-1988.

Vice-President Elect, Neurotoxicology Specialty of Society of Toxicology, 1986-1987.

Councilor, Neurotoxicology Specialty Section of Society of Toxicology, 1982-1985; 1989-1991.

## EXTERNAL ADVISORY BOARD MEMBER

Rutgers Environmental and Occupational Health Sciences Institute, External Committee, April, 2004 – 2007.

## EXTERNAL PROGRAM REVIEW COMMITTEES

Texas A & M University, External Toxicology Site Review Committee, November, 1998.

## INVITED PARTICIPANT FOR TELEVISION AND RADIO PROGRAMS

November 2007: Interviewed by Houston TV Channel 2 and 49 about lead exposure
http://www.criticalmention.com/vq/uofh/2007-09-05_cbs-hou_1713.wmv

November 2007: Interviewed by Houston Radio Station KTRH about lead exposure

August 2004: Interviewed by Houston TV Channel 2 for toxic fume exposure to industrial workers and neighborhoods (Resins, alcohols and industrial chemicals)

February 2002: Interviewed by several local Houston TV and radio stations for toxic fume exposure to industrial workers and neighborhoods (Metals, alcohols and industrial chemicals)

December, 2001: Interviewed by FOX TV local affiliate for toxic metal and chemical exposure to children resulting from chromated copper arsenate wooden playground equipment

February, 2001: NIH Mitochondrial Interest Group Video Conference. Organized by Steven J. Zullo, Laboratory of Biochemical Genetics, NIMH-NIH, Bethesda, MD.

September, 1991: Teleconference of University of Houston's Ocular Therapeutics Course. Houston, TX. Presented five lecture hours on Fundamentals of Ocular Pharmacology, Autonomic Pharmacology, and Hypnotics and Local Anesthetics.

July and August, 1991: Broadcast of my invited talk from First Annual Environmental Health Conference: Issues and Answers for the 1990's in the City of Houston. Sponsored by the Health and Human Services Department of the City of Houston. Houston, TX. Public Access Channel, Houston, TX.

September, 1985: Toxic Chemicals in the Environment. KUHF TV and Radio. Houston, TX. Round table discussion of effects and risks of environmental chemicals in the Gulf Coast Region.

November, 1980: Pesticides in the Environment: Friend or Foe? KUHF TV. Houston, TX. Presented summary of mechanisms of action and side effects and then answered phone calls.

## GRANT SUPPORT

### A. External

National Institute of Environmental Health Sciences (NIEHS), RO1 ES012482, 7/2003 to 7/2010, Prenatal Low-Level Lead Exposure and Retinal Toxicity, $1,798,315, Principal Investigator.

National Institute of Environmental Health Sciences (NIEHS), Research Supplement for Minority to RO1 ES012482-01, 7/2004 to 7/2006, Prenatal Low-Level Lead Exposure and Retinal Toxicity, $48, 545, Principal Investigator.

National Institute of Occupational Safety and Health (NIOSH) Environmental Research Center (ERC) Pilot Project Research Training Application, G090168, 9/2004 -8/2005, Retinal Toxicology of Lead-Exposed Children in Mexico City: Mechanistic Animal Research Studies, $10,000, Principal Investigator.

National Institute of Environmental Health Sciences (NIEHS), RO1 ES03183, 4/1992 to 4/1998, Lead Neurotoxicity in the Visual System, $819,374, Principal Investigator.

National Institute of Environmental Health Sciences (NIEHS), Research Supplement for Minority to RO1 ES03183, 10/1992 to 4/1996, Lead Neurotoxicity in the Visual System, $95,526, Principal Investigator.

National Institute of Environmental Health Sciences (NIEHS), RO1 ES03183, 5/84 to 5/90, Lead Neurotoxicity in the Visual System, $512,685, Principal Investigator.

National Institute of Environmental Health Sciences (NIEHS), RO1 ES 03183, 5/81 to 5/84, Lead Neurotoxicity in the Visual System, $177,204, Principal Investigator.

**B. Internal**

Institute for Biomedical Imaging Sciences, University of Houston-Methodist Hospital-Weill Cornell medical School Grant, 2007-2008. Metabolic Dysfunction in the Retina and Retinocortical Pathway: Biomarker for ALS, (b) (6), Principal Investigator.

Vision Research Support Grant, 2002-2003, Cytokine Signaling and Retinal Apoptosis. (b) (6), Principal Investigator.

University of Houston FERV Foundation, 1998-2003, (b) (6), Principal Investigator.

University of Houston PEER Grant, 1998-1999, Using Transgenic Mice to Examine the Role of Mitochondria and Bcl-xL in Lead-Induced Retinal Apoptosis. (b) (6), Principal Investigator.

Vision Research Support Grant, 1996-1997, Confocal Scanning Laser Microscopy Studies of Calcium Overload and Mitochondrial Dysfunction in Retina. (b) (6), Principal Investigator.

University of Houston PEER Grant, 1996-1997, Molecular Mechanisms Underlying the Dopamine-induced Inhibition of Retinal $Na^+,K^+$-ATPase. (b) (6) Principal Investigator.

Biomedical Research Support Grant, 1989-1990. Retinal cyclic GMP metabolism in lead-exposed rats: Cellular and molecular studies, (b) (6), Principal Investigator.

Biomedical Research Support Grant, 1988-1989, Cellular studies of the lead-induced selective rod degeneration in isolated rod photoreceptor cells, (b) (6), Principal Investigator.

Biomedical Research Support Grant, 1987-1988, Fetal Alcohol and the Visual System , (b) (6), Principal Investigator.

Biomedical Research Support Grant, 1983-1984, Quantitative Morphometrics in the Visual System, (b) (6), Principal Investigator.

New Research Opportunities Program, 1982-1983, Pharmacological Investigations of Visual Cortex, (b) (6), Principal Investigator.

Biomedical Research Support Grant, 1981-1982, Scotopic and Photopic Analyses of Retina, (b) (6), Principal Investigator.

University of Texas Education Grant, 1980-1981, Proposal for Improving Instruction in Toxicology, (b) (6), Principal Investigator.

**UNIVERSITY OF HOUSTON COMMITTEES**

**A. University Level**

Chair, Athletic Advisory Committee, 2006-2009
Member, Athletic Advisory NCAA Recertification Committee, 2004-2006.
Member, Athletic Advisory Committee, 2003-2006.
Chair of Subcommittee on Minority Equity Issues: 2003-2005.

Faculty Member on Cougar Pride: 2005-Present.
Chair, Intellectual Property Committee, 2004-2007.
Member, Intellectual Property Committee, 1989-1998; 2001-2004; 2007-Present.
Member, Substance Abuse Committee, 1999-2001.
Member, Biohazards Committee, 1997-Present.
Member, Research Excellence Committee, 1996-1997.
Member, Chemical Safety Committee, 1992-1995; 1996-Present.
Member, Graduate and Professional Council, 1984-1992.
Member, Task Force on Technology Transfer, 1988-1989.
Member, Search Committee for Head of Task Force on Technology Transfer, 1989-1990.
Member, Promotion and Tenure Committee, 1989-1990.
Member, Search Committee for Chairman of Biology Department, 1989-1990.
Member, Search Committee for Biology Department, 1991-1992; 1994-1996.
Chair, Graduate Admissions Committee, Graduate and Professional Council, 1988-1989.
Chair, Graduate Policy Committee, Graduate and Professional Council, 1985-1986.
Co-Chair, Course Review Committee, Graduate and Professional Council, 1987-1988.
Member, Ad Hoc Awards Committee for RIG, REG, NROP, BRSG: 1983-Present.
Member, Carcinogen Committee, 1984-1990.

**B. Optometry College Level**

Member, Research Planning Committee and Subcommittees, 1998-Present.
Chair, Faculty Recruitment Committee, 1996-1997.
Member, Faculty Nomination Committee, 1997-Present.
Chair, Faculty Review Committee, 1995-1996.
Member, Faculty Review Committee, 1994-1995.
Member, Graduate Curriculum Committee, 1995-Present.
Member, Faculty Salary Augmentation Committee, 1995-1996.
Member, Distinguished Professor Committee, 1995-1996.
Member, College Safety and Biohazards Committee, 1994-Present.
Chair, Graduate Student Admissions Committee, 1990-1992.
Member, Graduate Student Admissions Committee, 1989-2002.
Member, Ph.D. Qualifying Committee, 1989-Present.
Member, Faculty Affairs Committee, 1992-1994.
Member, Electron Microscopy Committee, 1992-Present.
Member, Faculty Recruitment Committee, 1988-1995; 2003-Present.
Member, Faculty Seminar Committee, 1987-1989; 1991-1995.
Chair, Budget Advisory Committee, 1986-1988.
Chair, Animal Care Committee, 1985-1987.
Chair, Graduate Curriculum Committee, 1983-1984.
Coordinator, Electron Microscopy/Histology Laboratory, 1986-1988.
Member, Graduate Curriculum Committee, 1982-Present.
Chair, Graduate Curriculum Committee, 1992-1995.
Member, Human Subjects Committee, 1983-1984, 1986-1987.
Member, Graduate Recruitment Committee, 1983-1989.
Member, Animal Care Committee, 1982-Present.
Member, Professional Student Scholarship Committee, 1982-1983; 1985-1986.

**C. Other Colleges at University of Houston**

Member, Promotion and Tenure Committee, College of Pharmacy, 1997, 2000, 2004.
Member, Faculty Search Committee, Biology and Biochemistry, 1994-1995; 2005-2007.

**ORGANIZED AND / OR CHAIRED NATIONAL AND INTERNATIONAL SYMPOSIA**

May, 2008: International Neurotoxicology Association (INA), Chair of Scientific Program Committee for INA-12 in Jerusalem, Israel 2009. (Organized meeting; Chaired and presented talk at Symposium on "Neurotoxicity and Alterations in Cellular Development").

March, 2008: Society of Toxicology, Nashville, TN. (Organized and Co-chaired Symposium entitled "Neurotoxicant Exposure Produces Persistent Alterations in Developmental and Adult Neurogenesis: Structure-Function Studies and Clinical Relevance.").

June, 2007: Eleventh International Neurotoxicology Association Meeting, Co-chair of Program Committee for entire Meeting, Asilomar, CA.

October-November, 2006: Co-organized all 18 retinal sessions for International Congress of Eye Research XI, Buenos Aires, Argentina.

July, 2005: Ninth International Neurotoxicology Association Meeting, Co-chair of Symposium entitled "Sensory Neurotoxicology". Helsinki, Finland.

May, 2004: Association for Research in Vision and Ophthalmology (ARVO), Ft. Lauderdale, FL. (Organized and Chaired Special Interest Symposium entitled "Calcium Compartmentation in Photoreceptors").

February, 2003: Association for Ocular Pharmacology and Therapeutics, Kona, Hawaii. (Co-chaired symposium on "Toxicology").

May, 2002: Association for Research in Vision and Ophthalmology (ARVO), Ft. Lauderdale, FL. (Co-organized and co-chaired Minisymposium entitled "Retinal Glia in Development, Injury and Regeneration"

March, 2002: Society of Toxicology, Nashville, TN. (Co-organized and Co-chaired Symposium entitled "Imaging the Mitochondrial Permeability Transition Pore in Toxic Injury").

July, 2001: Eighth International Neurotoxicology Association Meeting, Co-chaired Symposium entitled "Developmental Neurotoxicology". Lisbon, Portugal.

May, 2001: Association for Research in Vision and Ophthalmology (ARVO), Ft. Lauderdale, FL. (Organized and will Chair Sunday Plenary Symposium entitled "Recent Advances in the Molecular Mechanisms of Apoptotic Cell Death and Cytoprotection in the Eye: A Symposium Dedicated to Dr. Richard N. Lolley").

March, 2001: Society of Toxicology, San Francisco, CA. (Organized and Co-chaired Symposium entitled "Ocular Toxicology and Apoptosis").

October, 2000: International Congress of Eye Research, Santa Fe, NM. (Organized and Chaired Symposium entitled: "Cellular and Molecular Mechanisms of Retinal Apoptosis).

October, 2000: International Congress of Eye Research, Santa Fe, NM. (Organized and Co-Chaired Symposium entitled: "Mechanisms of Regulating $Na^+$,$K^+$-ATPase Activity in Ocular Tissues).

May, 1999: Association for Research in Vision and Ophthalmology (ARVO), Ft. Lauderdale, FL. (Organized and Chaired Special Interest Symposium entitled "Retinal Apoptosis").

July, 1998: International Congress of Toxicology VIII, Paris, France. (Organized and Chaired Symposium entitled "Functional Consequences of Altered Gene Expression in Toxicology").

July, 1995: International Congress of Toxicology VII, Seattle, WA. (Organized and Chaired Continuing Education Course entitled "Cellular and Molecular Mechanisms of Apoptosis").

November, 1994: Gulf Coast Society of Toxicology, Houston, TX. (Organized Symposium entitled Endocrine Disrupters in the Environment).

September, 1992: 10th International Neurotoxicology Conference, Mechanisms of Developmental Neurotoxicology, Little Rock, AR. (Co-Organizer of Meeting and Chaired Session).

October, 1991: 9th International Neurotoxicology Conference, New Dimensions of Lead Toxicity: Redefining Mechanisms and Effects, Little Rock, AR. (Chaired Tuesday AM session).

October, 1990: 8th International Neurotoxicology Conference, Role of Toxicants In Neurological Disorders, Little Rock, AR. (Chaired afternoon session).

March, 1989: Society of Toxicology, 28th Annual Meeting, Neurotoxicant-Induced Alterations in Cell-Cell Communication Symposium, Atlanta, GA. (Organized and Chaired).

February, 1987: Society of Toxicology, 26th Annual Meeting, Neurotoxicology of Heavy Metals Symposium, Washington, D.C. (Co-organized).

November, 1986: Society for Neuroscience, 16th Annual Meeting, Neurobiological Mechanisms of Toxicant-Induced Axonopathies Symposium, Washington, D.C. (Co-organized and Chaired).

March, 1985: Society of Toxicology, 23rd Annual Meeting, Sensory System Toxicology: An Interdisciplinary Approach, San Diego, CA. (Organized and Chaired).

September, 1983: Food and Drug Administration, International Symposium entitled "Ocular Photoxicity and Light Exposure", Bethesda, MD (Organized and Chaired a Session).

**INVITED SPEAKER AT NATIONAL AND / OR INTERNATIONAL SYMPOSIA**

November, 2007: Twenty-Fourth International Neurotoxicology Conference, Environmental Etiologies of Neurological Disorders, San Antonio, TX.

October, 2007: 17th Lake Shirakaba Conference, Aurora in Regeneration, Tokai University European Center, Vedbaek, Denmark.

June, 2007: United Mitochondrial Disease Foundation: Mitochondrial Medicine 2007 Meeting, Symposium entitled "Mitochondrial Mechanisms and Disease". San Diego, CA.

October-November, 2006: International Congress of Eye Research XI, Symposium entitled "Mitochondria in Retinal Degeneration". Buenos Aires, Argentina.

October, 2006: Neuroprotective Strategies in Degenerative Eye Disease Symposium, Vanderbilt Eye Institute, Session "Degenerative Processes in the Outer Retina: Mechanisms and Interventions", Nashville, TN.

July, 2005: Tenth International Neurotoxicology Association Meeting, Symposium entitled "Sensory System Neurotoxicology". Helsinki (Haikoo), Finland.

June, 2005: Visual Function: Insights from Revolution in Biology at the Molecular Level Meeting, Symposium entitled "Light Processing: A Life and Death Story". Tel Aviv, Israel.

March, 2004: Society of Toxicology, Baltimore, MD. Symposium entitled "Use of Molecular Approaches to Examine Mechanisms of Neurotoxicity".

February, 2004: Twenty-First International Neurotoxicology Conference, Infant and Child Neurotoxicity Studies: Subtle and Long-Term Effects, Honolulu, Hawaii.

July, 2003: Ninth International Neurotoxicology Association Meeting, Symposium entitled "Visual System Neurotoxicology: Human and Animal Correlates". Dresden, Germany.

February, 2003: Association for Ocular Pharmacology and Therapeutics, Kona, Hawaii. Symposium entitled "Toxicology".

April, 2002: Keystone Symposia, Copper Mountain Resort, CO. Symposium entitled "Mitochondria and Pathogenesis".

March, 2002: Society of Toxicology, Nashville, TN. Symposium entitled "Imaging the Mitochondrial Permeability Transition Pore in Toxic Injury".

February, 2002: Workshop on Neuroprotection in Ophthalmology: From Basic Research to New Therapeutic Strategies for Eye Disease. University of Rome Tor Vergata Medical School, Rome, Italy.

July, 2001: Eighth International Neurotoxicology Association Meeting, Symposium entitled "Developmental Neurotoxicology". Lisbon, Portugal.

May, 2001: Association for Research in Vision and Ophthalmology (ARVO), Ft. Lauderdale, FL. Sunday Plenary Symposium entitled "Recent Advances in the Molecular Mechanisms of Apoptotic Cell Death and Cytoprotection in the Eye: A Symposium Dedicated to Dr. Richard N. Lolley".

March, 2001: Society of Toxicology, San Francisco, CA. Symposium entitled "Ocular Toxicology and Apoptosis".

October, 2000: International Congress of Eye Research, Santa Fe, NM. Symposium entitled "Cellular and Molecular Mechanisms of Retinal Apoptosis".

April, 2000: EuroConference on New Cellular Targets of Cytotoxic Mechanisms, San Feliu de Guixols, Spain. Symposium entitled "Molecular Mechanisms of Neuronal Apoptosis". (Declined)

July, 1999: Seventh International Neurotoxicology Association Meeting, Symposium entitled "Sensory System Toxicology'. Leicester, United Kingdom.

May, 1999: Association for Research in Vision and Ophthalmology (ARVO), Ft. Lauderdale, FL. Special Interest Symposium on Retinal Apoptosis.

November, 1998: Fourth International Congress on Neuroprotective Agents, Annapolis, MD. Symposium entitled "Apoptosis in the Nervous System". (Declined).

July, 1998: Eighth International Congress of Toxicology, Paris, France. Symposium entitled "Functional Consequences of Altered Gene Expression in Toxicology".

October, 1997: International Conference on Volatile Organic Compounds: Neurotoxicology and Risk Assessment, University of Pavia, Italy (Declined).

September, 1997: Fifth National Board for Laboratory Animals (CFN) Meeting, Stockholm, Sweden. Symposium entitled "Effects of Toxicants on Gene Expression and Intracellular Signalling".

October, 1996: Fifth International Society of Ocular Toxicology, Asheville, North Carolina.

September, 1995: Fifth International Symposium on Environmental Neurotoxicology: From Molecular Biology to Behavior. Dusseldorf, Germany.

July, 1995: FASEB Summer Research Conference on Biology and Chemistry of Vision, Copper Mountain, CO.

July, 1995: Seventh International Congress of Toxicology, Cellular and Molecular Mechanisms of Neurotoxicolgy Symposium, Seattle, WA.

May, 1995: 66th Annual Association for Research in Vision and Ophthalmology (ARVO) Meeting, Symposium on Mechanisms of Apoptosis, Ft. Lauderdale, FL.

April/May, 1995: International Conference on Drugs of Abuse, Funchal - Madeira, Portugal.

November, 1994: VI International Symposium on Retinal Degeneration. Jerusalem, Israel.

May, 1994: 65th Annual Association for Research in Vision and Ophthalmology (ARVO) Meeting, Symposium on Mechanisms of Cell Death: Suicide or Assassination, Sarasota, FL.

July, 1993: Eleventh International Biophysics Congress, Satellite Symposium on Heavy Metal Induced Alterations in Excitable Membranes, Tihany, Hungary.

June, 1993: Third International Neurotoxicology Association Meeting, Cellular Signaling and Second Messengers in Neurotoxicology, Copenhagen, Denmark.

October, 1991: Ninth International Neurotoxicology Conference, New Dimensions of Lead Toxicity: Redefining Mechanisms and Effects, Little Rock, AR.

June, 1991: First Annual Environmental Health Conference: Issues and Answers for the 1990's in the City of Houston. Sponsored by the Health and Human Services Department of the City of Houston. Houston, TX.

October, 1990: Eighth International Neurotoxicology Conference, Role of Toxicants in Neurological Disorders, Little Rock, AR.

October, 1990: International Symposium on Toxicology, Beijing, China. (declined)

May, 1989: Second International Neurotoxicology Association Meeting, Sensory Systems as Targets for Neurotoxic Agents Symposium, Sitges, Spain.

March, 1989: Society of Toxicology, 28th Annual Meeting, Neurotoxicant-Induced Alterations in Cell-Cell Communication Symposium, Atlanta, GA.

September, 1988: Eighth International Congress of Eye Research, Retinal Degeneration Symposium, San Francisco, CA.

February, 1987: Society of Toxicology, 26th Annual Meeting, Neurotoxicology of Heavy Metals Symposium, Washington, D.C.

November, 1986: Society for Neuroscience, 16th Annual Meeting, Neurobiological Mechanisms of Toxicant-Induced Axonopathies Symposium, Washington, D.C.

December, 1985: International Meeting for Assessing Neurobehavioral Methods in Safety Assessment of Chemicals and Drugs, Dusseldorf, Germany.

October, 1985: University of Cincinnati Medical Center, Department of Environmental Health, Kettering Laboratory, Honorary Symposium for Dr. Raymond Suskind, Cincinnati, OH.

March, 1985: Society of Toxicology, 23rd Annual Meeting, Sensory System Toxicology: An Interdisciplinary Approach, San Diego, CA.

March, 1984: Society of Toxicology, 22nd Annual Meeting, Experimental Models of Toxicant-Induced Axonopathies, Atlanta, GA.

September, 1983: Food and Drug Administration, International Symposium on Ocular Photoxicity and Light Exposure, Bethesda, MD

August, 1983: International Congress on Toxicology III, Cellular and Molecular Neurotoxicity Symposium, San Diego, CA.

March, 1982: International Brain Research Organization, Satellite Symposium on Neurotoxicology of Environmental Pollutants, Dusseldorf, Germany.

June, 1982: Teratology Society, Behavioral Teratology Subsection, Symposium on Heavy Metal Neurotoxicity, Atlantic City, NJ.

April, 1979: U. S. Environmental Protection Agency, International Symposium on Behavioral Test Methods for Neurotoxic Substances, San Antonio, TX.

**INVITED SEMINAR SPEAKER (Selected from 1990-Present)**

January, 2009: University of Tennessee Health Science Center, College of Medicine, Department of Ophthalmology, Vision Science Seminar Series, Memphis, TN.

November, 2008: Rutgers University College of Medicine and Dentistry, Environmental & Occupational Health Sciences Institute, Toxicology Seminar Series, Piscataway, NJ.

February, 2008: University of Florida, Center for Vision Research Seminar Series, Gainesville, FL.

September, 2007: University of Pennsylvania, Neuroscience Seminar Series, Philadelphia, PA.

June, 2007: National Institute of Environmental Health (NIEHS), Research Triangle Park, NC RTP, NC.

March 2006: University of California: San Diego, Department of Structural Biology, San Diego, CA.

June, 2005: Shaare Zedek Medical Center, Department of Ophthalmology and Department of Neurology, Jerusalem, Israel.

April, 2005: St. Louis University School of Medicine, Department of Pharmacology and Physiological Science, St. Louis, MO.

October, 2003: Leicester, England, Medical Research Council on Toxicology, University of Leicester,, England.

October, 2003: Baylor College of Medicine, Huffington Center on Aging Seminar Series, Houston, TX.

April, 2003: University of Houston, Department of Pharmacology, Houston, TX.

May, 2002: University of Texas Medical School: Houston, Department of Molecular Pathology Seminar Series.

April, 2002: Karolinska Institute, Institute of Environmental Medicine, Department of Toxicology. Stockholm, Sweden.

February, 2002: University of Padua, Departments of Biochemistry and Environmental Medicine, Padua, Italy.

February, 2001: NIH Mitochondrial Interest Group Video Conference. Organized by Steven J. Zullo, Laboratory of Biochemical Genetics, NIMH-NIH, Bethesda, MD.

November, 1999: Texas A & M University System Health Science Center, Department of Medical Pharmacology and Toxicology, College of Medicine, Toxicology Seminar Series, College Station, TX.

May, 1999: Baylor College of Medicine, Cardiovascular Science Seminar Series, Houston, TX.

April, 1999: Department of Pharmacology and Pharmaceutical Sciences, University of Houston: Pharmacology Seminar Series. Houston, TX.

January, 1999: University of Gothenberg, Gothenberg, Sweden.

April, 1998: University of Texas Medical Center, School of Public Health. Houston, TX.

September, 1997: University of Manchester, School of Biological Sciences, Department of Pharmacology and Toxicology, Manchester, England.

April, 1997: Department of Pharmacology and Pharmaceutical Sciences, University of Houston: Pharmacology Seminar Series. Houston, TX.

November, 1996: Karolinska Institute, Institute of Environmental Medicine, Department of Toxicology. Stockholm, Sweden.

May, 1996: University of Cincinnati Medical Center, Department of Environmental Health, Cincinnati, OH.

May, 1996: Wayne State University, Institute of Chemical Toxicology and NIEHS Center in Molecular and Cellular Toxicology, Detroit, MI.

September, 1995: The University of Goteberg, Zoology Institute, Goteberg, Sweden.

August, 1995: Alcon Laboratories, Fort Worth, TX.

March, 1995: The University of Pennsylvania, Institute for Environmental Studies, Philadelphia, PA.

October, 1993: The University of Connecticut, Center for Biochemical Toxicology. Storrs, CT.

July, 1993: University of Vienna, Institute of Tumorbiology. Vienna, Austria.

June, 1993: Karolinska Institute, Institute of Environmental Medicine, Department of Toxicology. Stockholm, Sweden.

June, 1993: National Institute of Occupational Safety and Health. Oslo, Norway.

December, 1992: The University of Texas Medical School at Houston Toxicology Seminar Series. Houston, TX.

November, 1992: The University of Texas Medical School at Galveston Toxicology Seminar Series. Galveston, TX.

July, 1991: University of Pavia Medical School, Department of Internal Medicine, Division of Pharmacology and Toxicology. Pavia, Italy.

July, 1991: University of Padua Medical School, Department of Environmental Medicine. Padua, Italy.

November, 1990: Department of Pharmacology, University of Houston: Pharmacology Seminar Series. Houston, TX.

October, 1990: Texas A & M University, Department of Veterinary Physiology and Pharmacology: Environmental Toxicology and Pharmacology Seminar Series. College Station, TX.

March, 1990: Baylor College of Medicine: Neuroscience Seminar Series. Houston, TX.

## TEACHING

A. Professional (O.D., Pharm.D. or M.D. Degree) Courses (* Denotes Course Organizer and Coordinator)

| | | |
|---|---|---|
| Nutrition and the Eye(*co-org.) | Optometry | 2000-Present |
| Molecular Mechanisms of Neurodegeneration* | Optometry | 1999-Present |
| General Pharmacology* | Optometry | 1981-Present |
| Ocular Pharmacology* | Optometry | 1986-Present |
| General Pathology | Optometry | 1990-2000 |
| Clinical Rounds and New Developments | Optometry | 1990-1991 |
| Ocular Physiology | Optometry | 1981-1985 |
| Neurotoxicology | Pharmacy | 1985-1989 |
| Environmental Optometry | Optometry | 1982-1988 |
| General Pharmacology | U.T. Medical School | 1979-1981 |
| Neuropharmacology | U.T. Medical School | 1979-1981 |
| Industrial Toxicology and Environmental Medicine* | U.T. Medical School | 1979-1981 |

B. Graduate Courses (* Denotes Course Organizer and Coordinator)

| | | |
|---|---|---|
| Molecular Genetics of Eye Disease | Graduate | 2000-Present |
| Biochemical / Cellular Mechanisms of Cell Injury and Death* | Graduate | 1998; 1999 |
| Adv. Seminar: Cellular / Molecular Mechanisms of Cell Death* | Graduate | 1994-95; 1995-96; 1996-98 |
| Adv. Seminar: Cellular and Molecular Retinal Development* | Graduate | 1993-94; 1996-97 |
| Adv. Seminar: Scotopic Vision* | Graduate | 1990-1991 |
| Rod-Cone Interactions* | Graduate | 1989-1990 |
| Neurochemistry | Biochemistry | 1986-7; 1988-9; 1990-91 |
| Basic Visual Neuroanatomy and Biochemistry* | Graduate | 1984-Present |
| Basic Visual Physiology and Neurophysiology* | Graduate | 1984-Present |
| Histology/Electron Microscopy* | Graduate | 1987-1988 |
| Advanced Neurophysiology | Graduate | 1986-1987 |
| Retinogeniculate Anatomy and Physiology* | Graduate | 1984-1985 |
| Seminar in Neurophysiology* | Graduate | 1982-1984 |
| Seminar in Physiological Optics* | Graduate | 1982-1983, 1989-90 |
| Laboratory Instrumentation and Techniques | Graduate | 1982-1984 |
| Neuropharmacology | U.T. Medical School | 1979-1981 |

| | | |
|---|---|---|
| Introduction to Toxicology | U.T. GSBS | 1979-1981 |
| Sites and Mechanisms of Toxicity* | U.T. GSBS | 1979-1981 |
| Chemical Contamination of the Environment | U.T. GSBS | 1979-1981 |
| Neurotoxicology Seminar* | U.T. GSBS | 1979-1981 |

C. Continuing Education Lectures

| | |
|---|---|
| Neurotoxicology: Curt Klaassen's Mid-America Toxicology Course (2 hours) | 1994-Present |
| Ocular Toxicology: Curt Klaassen's Mid-America Toxicology Course (1 hour) | 1995-Present |
| CNS Pharmacology and Toxicology: UHCO (6 hours per lecture) | 1987-1999 |
| Autonomic Pharmacology: UHCO | 1990-1999 |
| Advanced Neurotoxicology: Society of Toxicology (Organized and Chaired) | 1991 |
| Concepts in Cell Biology: Society of Toxicology (Organized) | 1990 |
| Fundamentals of Ocular Pharmacology: Texas Assoc. Optometrists | 1991 |
| Autonomic Pharmacology: Texas Assoc. Optometrists | 1991 |
| The Effects of Pharmaceuticals Upon the Eye: LSU Eye Center | 1989 |
| Fundamentals of Ocular Pharmacology and Toxicology: UHCO (4 hours) | 1987-2000 |
| Ocular Toxicology: UHCO | 1987-94, 2005 |

D. Proctor for Continuing Education Exams

| | |
|---|---|
| Pennsylvania College of Optometry: Remote Site Proctor | 1991-1995 |

E. Question Writer for National Board of Examiners of Optometry

| | |
|---|---|
| General Pharmacology: Basic Principles | 1989-1995 |
| Autonomic Pharmacology | 1989-1995 |
| Ocular Pharmacology: Basic Principles | 1989-1995 |

F. Judge for Manuscript Awards

| | |
|---|---|
| Mechanisms Specialty Section of Society of Toxicology 20th Annual Student Awards for Meritorious Research (reviewed and ranked 12 full-length manuscript) | 2001-2002 |
| U.S. Environmental Protection Agency Neurotoxicology Division Scientific Achievement on Neurotoxicity (SAINT) Award (reviewed 8 full-length manuscripts and ranked the top-three) | 2001-2002 |

## GRADUATE STUDENTS, POSTDOCTORAL FELLOWS AND VISITING SCIENTISTS

A. Ph.D. Thesis Committees and Oral Exam (and University affiliation)

(b) (6)

| | | |
|---|---|---|
| U.H. Biology | 2009-Present | Advisor |
| U.H. Biochemistry | 2006-Present | Advisor |
| U.H. Biochemistry | 2005-Present | Advisor |
| U.H. Biochemistry | 2005-2007 | Advisor |
| U.H. Biochemistry | 2005-Present | Advisor |
| U.H. Biochemistry | 2003-2008 | Advisor |
| U.H. Biochemistry | 2002-2005 | Committee |
| U.H. Biochemistry | 1999-2003 | Committee |
| U.H. Biochemistry | 1994-2000 | Advisor |
| U.H. Biochemistry | 1997-2005 | Committee |
| U.H. Biology | 1996-2001 | Committee |
| U.H. Pharmacology | 1998-1999 | Committee |
| UHCO | 1992-1998 | Advisor |
| U.H. Biochemistry | 1995-1999 | Advisor |
| U.H. Biochemistry | 1991-1996 | Advisor |
| UHCO | 1988-1994 | Advisor |
| UHCO | 1987-1994 | Advisor |
| UHCO | 1987-1989 | Consultant |
| UHCO | 1983-1987 | Advisor |
| UTHSC | 1980-1983 | Committee |
| UTHSC | 1979-1981 | Committee |

B. Master's Thesis Committees and Oral Exam (and University affiliation)

(b) (6)

| | | |
|---|---|---|
| UHCO | 1997-1998 | Committee |
| U.H. Biology | 1994-1997 | Committee |
| UHCO | 1994-1995 | Advisor |
| UHCO | 1993-1995 | Advisor |
| U.H. Biochemistry | 1991-1992 | Committee |
| UTHSC | 1980-1983 | Committee |

C. Honors Undergraduate Thesis (and University affiliation) & Undergraduate Students with NIH Grants

(b) (6)

| | | |
|---|---|---|
| U.H. Biology | 2004-2006 | Advisor |
| U.H. Biology | 1997-1998 | Advisor |
| U.H. Biology | 1997-1998 | Advisor |

D. Graduate Students in Physiological Optics and Visual Science Program or Biology and Biochemistry

(b) (6)

| | | |
|---|---|---|
| U.H. Biology | 2009-Present | Advisor |
| Ph.D. | 2006-Present | Advisor |
| Ph.D. | 2005-Present | Advisor |
| Ph.D. | 2005-2007 | Advisor |

| | | | |
|---|---|---|---|
| (b) (6) [redacted] | Ph.D. | 2005-Present | Advisor |
| | Ph.D. | 2003-2008 | Advisor |
| | Ph.D. | 1994-2000 | Advisor |
| | Ph.D. | 1995-1999 | Advisor |
| | Ph.D. | 1992-1998 | Advisor |
| | Ph.D. | 1991-1996 | Advisor |
| | M.S. | 1993-1995 | Advisor |
| | Ph.D. | 1988-1994 | Advisor |
| | M.S. | 1993-1995 | Advisor |
| | Ph.D. | 1987-1994 | Advisor |
| | Ph.D. | 1988-1990 | Advisor |
| | Ph.D. | 1983-1987 | Advisor |

E. Postdoctoral Fellows (Funded by RO1 Grants)

| | |
|---|---|
| (b) (6) [redacted] | 2004-2007 |
| | 2003-2005 (NEI Postdoctoral Fellow 2003-2004) |
| | 1994-1996 (NEI Postdoctoral Fellow 1994-1995) |
| | 1994-1995 |
| | 1981-1983 |
| | 1981-1982 |

F. Research Assistant Professorships (Funded by RO1 Grants)

| | |
|---|---|
| (b) (6) [redacted] | 2003-2005 |
| | 2007-Present |

G. Visiting Scholars (Funded by RO1 Grants)

| | |
|---|---|
| (b) (6) [redacted] | 1985-1988 |

H. National Eye Institute (NEI) Summer Fellows (Funded by NEI Training Grant)

| | |
|---|---|
| (b) (6) [redacted] | 1999-2000 |
| | 1998-1999 |
| | 1997-1998 |
| | 1994-1997 |
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| | 1987-1988 |
| | 1986-1987 |
| | 1985-1987 |

I. Research and Travel Awards to Students

| | | |
|---|---|---|
| 2009 | (b) (6) [redacted] | ARVO Travel Award, Cutaneous and Ocular Toxicology, 48th Annual Meeting in Ft. Lauderdale, FL. |
| 2008 | [redacted] | SOT Neurotoxicology Best Poster Award, 47th Annual Society of Toxicology Meeting in Seattle, WA. |
| 2008 | [redacted] | SOT Neurotoxicology Graduate Student Award for Poster Presentation at 47th Annual Society of Toxicology Meeting in Seattle, WA. |
| 2007 | [redacted] | SOT Neurotoxicology Graduate Student Award for Poster Presentation at 46th Annual Society of Toxicology Meeting in Charlotte, NC. |
| 2007 | [redacted] | SOT Graduate Student Travel Award to 46th Annual Society of Toxicology Meeting in Charlotte, NC to present his poster. |
| 2007 | [redacted] | Alliance for Graduate Education and the Professoriate (AGEP) program Summer Research Award. |
| 2006 | [redacted] | Alliance for Graduate Education and the Professoriate (AGEP) program Summer Research Award. |
| 2006 | [redacted] | Pfizer Travel Award for Undergraduate Research: 45th Annual Society of Toxicology Meeting, San Diego, CA. |
| 2004 | [redacted] | Finalist for Graduate Student Award: 43th Annual Society of Toxicology Meeting, Mechanisms Specialty Section, Baltimore, MD. |
| 2000 | [redacted] | Travel Award to 3rd Albany Conference on Frontiers of Mitochondrial Research, Rensselaerville, NY. |
| 1998 | [redacted] | Basic Research and Academic Initiatives in Neuroscience (BRAIN) Graduate Student Travel Award for the 37th Annual Society of Toxicology Meeting, Seattle, WA. |
| 1998 | [redacted] | Graduate Student Award: Third Place at 37th Annual Society of Toxicology Meeting, In Vitro Specialty Section, Seattle, WA. |
| 1997 | [redacted] | Basic Research and Academic Initiatives in Neuroscience (BRAIN) Graduate Student Travel |

Award for the 36th Annual Society of Toxicology Meeting, , Cincinnati, OH.

(b) (6)

1997 Graduate Student Award: Runner-Up at 36th Annual Society of Toxicology Meeting, Neurotoxicology Specialty Section, Cincinnati, OH.

1997 Basic Research and Academic Initiatives in Neuroscience (BRAIN) Graduate Student Travel Award for the 36th Annual Society of Toxicology Meeting, , Cincinnati, OH.

1997 Finalist for Graduate Student Award: 36th Annual Society of Toxicology Meeting, Mechanisms Specialty Section, Cincinnati, OH.

1996 Semi-Finalist for Graduate Student Award: 35th Annual Society of Toxicology Meeting, Mechanisms Specialty Section, Anaheim, CA.

1994 First Place Poster Award at Sigma Xi Science Day. University of Houston, Houston, TX.

1994 Fourth Place Graduate Student Award: 33rd Annual Society of Toxicology Meeting, Mechanisms Specialty Section, Dallas, TX.

1994 ARVO / Retina Research Foundation Lawrence Travel Fellowship Grant for the 1994 Annual Meeting, Sarasota, FL.

1993 Second Place for Platform Presentation: Gulf Coast Society of Toxicology Annual Meeting, Galveston, TX.

1992 Honorable Mention Graduate Student Award: 31st Annual Society of Toxicology Meeting, Mechanisms Specialty Section, Seattle, WA.

1992 Meritorious Poster Award: Annual Sigma XI Poster Day, University of Houston, Houston, TX.

1992 Third Place Graduate Student Award: 31st Annual Society of Toxicology Meeting, Neurotoxicology Specialty Section, Seattle, WA.

1992 SOT Graduate Student Travel Award to 31st Annual Society of Toxicology Meeting in Seattle, WA to present his poster.

(b) (6)

| | |
|---|---|
| 1991 | First Place Graduate Student Award: 30th Annual Society of Toxicology Meeting, Neurotoxicology Specialty Section, Dallas, TX. |
| 1990 | Fight for Sight Summer Fellowship Research Award |
| 1990 | Sigma Xi Research Grant |
| 1990 | Sigma Xi Research Grant |
| 1989 | First Place Graduate Student Award: 28th Annual Society of Toxicology Meeting, Neurotoxicology Specialty Section, Atlanta, GA. |
| 1986 | Sigma Xi Research Grant and Beta Sigma Kappa Grant. |
| 1984 | First Place Stella Ehrhardt Memorial Fund Cullen Graduate Fellowship at University of Houston. |
| 1983 | Sigma Xi Research Grant and First Place Poster Award at Sigma Xi Science Day. |

J. Minority Mentoring Program for Undergraduates

| | |
|---|---|
| 1996 | Society of Toxicology, 35th Annual Meeting, Anaheim, CA |
| 1997 | Society of Toxicology, 36th Annual Meeting, Cincinnati, OH |
| 1998 | Society of Toxicology, 37th Annual Meeting, Seattle, WA |
| 1999 | Society of Toxicology, 38th Annual Meeting, New Orleans, LA |

**PEER-REVIEWED IMAGES and VIDEOS: American Society Cell Biology**

1. Perkins G.A., Ellisman M.H. and **Fox D.A.** Mitochondrion in a Rod Photoreceptor Spherule. ASCB Image and Video Library. 2008; CYT-157. Available at: http://cellimages.ascb.org/cdm4/item_viewer.php?CISOROOT=/p4041coll12&CISOPTR=291&CISOBOX=1&REC=6

2. Perkins G.A., Ellisman M.H. and **Fox D.A.** Mitochondrion in a Cone Photoreceptor Pedicle. ASCB Image and Video Library. 2008; CYT-158. Available at:: http://cellimages.ascb.org/cdm4/item_viewer.php?CISOROOT=/p4041coll12&CISOPTR=292&CISOBOX=1&REC=4

3. Perkins G.A., Ellisman M.H. and **Fox D.A.** Mitochondrion in a Rod Photoreceptor Spherule. ASCB Image and Video Library. 2008; VID-92. Available at: http://cellimages.ascb.org/cdm4/item_viewer.php?CISOROOT=/p4041coll12&CISOPTR=352&CISOBOX=1&REC=7.

4. Perkins G.A., Poquiz P.H., Ellisman M.H. and **Fox D.A.** Mitochondrion in a Cone Photoreceptor Pedicle. ASCB Image and Video Library. 2008; VID-93. Available at: http://cellimages.ascb.org/cdm4/item_viewer.php?CISOROOT=/p4041coll12&CISOPTR=353&CISOBOX=1&REC=5

## PEER-REVIEWED PUBLICATIONS


1. **Fox D.A.**, Lewkowski J.P. and Cooper G.P. (1977). Acute and chronic effects of neonatal lead exposure on the development of the visual evoked response in rats. Toxicol. Appl. Pharmacol. 40: 449-461.

2. Bornschein R.L., **Fox D.A.** and Michaelson I.A. (1977). Estimation of the daily exposure in neonatal rats receiving lead via dams' milk. Toxicol. Appl. Pharmacol. 40: 577-587.

3. **Fox D.A.**, Overmann S. R. and Woolley D.E. (1979). Neurobehavioral ontogeny in lead-exposed rats. I. Reflex development and somatic indices. Neurotoxicology 1: 125-147.

4. **Fox D.A.**, Overmann S.R. and Woolley D.E. (1979). Neurobehavioral ontogeny in lead-exposed rats. II. Maximal electroshock seizures in developing and adult rats. Neurotoxicology 1: 149-170.

5. **Fox D.A.**, Lewkowski J.P. and Cooper G.P. (1979). Persistent visual cortex excitability alterations produced by neonatal lead exposure. Neurobehav. Toxicol. 1: 101-106.

6. **Fox D.A.** (1979). Physiological and neurobehavioral alterations during development in lead-exposed rats. Neurobehav. Toxicol. Teratol. 1: Suppl. 1, 193-206.

7. **Fox D.A.** and Sillman A.J. (1979). Heavy metals affect rod, but not cone, photoreceptors. Science 206: 78-80.

8. **Fox D.A.** (1979). Physiological and neurobehavioral alterations during development in lead-exposed rats. Neurobehav. Toxicol. Teratol. 1: 193-206.

9. Kendall D.A., **Fox D.A.** and Enna S.J. (1981). Effects of gamma-vinyl GABA on bicuculline-induced seizures. Neuropharmacology 20: 351-355.

10. **Fox D.A.**, Lowndes, H.E. and Bierkamper G.G. (1981). Electrophysiological test methods in neurotoxicology. In: *Target Organ Toxicity The Nervous System.* Ed. Mitchell, C. L., Raven Press, New York, 299-335.

11. **Fox D.A.**, Wright A.A. and Costa L.G. (1982). Visual acuity deficits following neonatal lead exposure: Cholinergic interactions. Neurobehav. Toxicol. Teratol. 4 : 689-693.

12. **Fox D.A.** (1982). Pharmacological and biochemical evaluation of triethyltin's anticonvulsant effects. Neurobehav. Toxicol. Teratol. 4: 273-278.

13. Doctor S.V. and **Fox D.A.** (1982). Effects of organotins on maximal electroshock seizure responsiveness in mice: I. Tri(n-alkyl)tins. J. Toxicol. Environ. Hlth. 10: 43-52.

14. Doctor S.V. and **Fox D.A.** (1982). Effects of organotins on maximal electroshock seizure responsiveness in mice: II. Tricyclohexyltin and triphenyltin. J. Toxicol. Environ. Hlth. 10: 52-58.

15. Doctor S.V. and **Fox D.A.** (1982). On the role of carbonic anhydrase in the anticonvulsant effects of triethyltin. Experientia 38: 824-825.

16. Costa L.G. and **Fox D.A.** (1983). A selective decrease in cholinergic muscarinic receptors in the visual cortex of adult rats following developmental lead exposure. Brain Res. 276: 259-266.

17. **Fox D.A.** and Doctor S.V. (1983). Triethyltin decreases maximal electroshock seizure in adult rats. Toxicol. Appl. Pharmacol. 68: 260-267.

18. Doctor S.V. and **Fox D.A.** (1983). Immediate and long-term alterations in maximal electroshock seizure responsiveness in rats neonatally exposed to triethyltin. Toxicol. Appl. Pharmacol. 68: 268-281.

19. **Fox D.A.** (1984). Ocular drug photoxicity. Intraocular Insights 1: 1-4.

20. **Fox D.A.** (1987). Electrophysiological alterations in humans and animals following low-level developmental lead exposure . Fund. Appl. Toxicol. 9 : 599-606.

21. **Fox D.A.** and Farber D.B. (1988). Rods are selectively altered by lead: I. Electrophysiology and biochemistry. Exp. Eye Res. 46: 597-611.

22. **Fox D.A.** and Chu L.W.-F. (1988). Rods are selectively altered by lead: II. Ultrastructure and quantitative histology. Exp. Eye Res. 46: 613-625.

23. **Fox D.A.** and Rubinstein S.D. (1989). Age-related changes in retinal sensitivity, rhodopsin content and rod outer segment length in hooded rats following low-level lead exposure during development. Exp. Eye Res. 48: 237-249.

24. **Fox D.A.** and Ruan D.-Y. (1989). Time-dependent and frequency-dependent effects of potassium channel blockers on large and medium diameter optic tract axons. Brain Res. 498: 229-242.

25. **Fox D.A.**, Rubinstein S.D. and Hsu, P. (1991). Developmental lead exposure inhibits adult rat retinal, but not kidney, $Na^+,K^+$-ATPase. Toxicol. Appl. Pharmacol. 109: 482-493. **(Awarded Best Research Paper of 1991 published in either of the official SOT journals: Toxicology and Applied Pharmacology or Fundamental and Applied Pharmacology)**

26. Katz L.M. and **Fox D.A.** (1991). Prenatal ethanol exposure alters scotopic and photopic components of adult rat ERG. Investig. Ophthalmol. Vis. Sci. 32: 2861-2872.

27. **Fox D.A.**, Katz L.M. and Farber D.B. (1991). Low-level developmental lead exposure decreases the sensitivity, amplitude and temporal resolution of rods. Neurotoxicology 12: 641-654.

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29. **Fox D.A.** and Katz L.M. (1992). Developmental lead exposure selectively alters the scotopic ERG component of dark and light adaptation and increases rod calcium content. Vision Res. 32: 249-255.

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39. **Fox D.A.**, Campbell M.L. and Blocker Y.S. (1997). Functional alterations and apoptotic cell death in the retina following developmental or adult lead exposure. Neurotoxicology 18: 645-665.

40. **Fox D.A.**, Srivastava D., Poblenz A.T. and He L. (1998). Lead-induced alterations in gene expression and activity of retinal cGMP PDE results in calcium overload and rod selective apoptosis. Toxicol. In Vitro 12: 587-598.

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54. Perkins G.A., Ellisman M.H. and **Fox, D.A.** (2004). The structure-function correlates of mammalian rod and cone photoreceptor mitochondria: Observations and unanswered questions. Mitochondrion 4: 695-703, 2004.

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58. **Fox D.A.**, Kala S.V., Hamilton, W.R., Johnson J.E., O'Callaghan J.P. (2008) Low-level human equivalent gestational lead exposure produces supernormal scotopic electroretinograms, increased retinal neurogenesis and decreased dopamine utilization in rats. Environ. Hlth. Perspect. 116: 618-625.

59. **Fox D.A.**, Opanashuk L., Zharkovsky A., Weiss, B. (2009) Gene-chemical interactions in the developing mammalian nervous system: Effects on proliferation, neurogenesis and differentiation. NeuroToxicology (accepted).

60. Giddabasappa A., Johnson J.E., Xiao W., Chaney S., and **Fox D.A.** Low-level gestational lead exposure switches cell fate and increases proliferation of retinal progenitor cells in developing mouse retina. (In preparation).

61. Johnson J.E., Giddabasappa A., Chaney S., O'Callaghan J.P., Miller D.B.. and **Fox D.A.**. Low-level gestational lead exposure produces a selective decrease in mouse retinal dopaminergic amacrine cell density and distribution and alters dopaminergic metabolism. (In preparation).

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63. Xiao W., Giddabasappa A. Chaney S., and **Fox D.A.**. Gene and protein expression in the developing mouse retina during low-level gestational lead exposure. (In Preparation).

64. Johnson J.E., Perkins G.A., Brown J., Lahsaei P., Ghassemzadeh S., Ellisman M.H., and **Fox D.A.**. A three-dimensional electron tomographic analysis of rod photoreceptor synaptic terminal mitochondria in control, lead-exposed, and/or Bcl-xL transgenic mice. (In Preparation).

**BOOK CHAPTERS**

1. Cooper G.P., **Fox D.A.**, Howell W.E., Laurie R.D., Tsang W. and Lewkowski J.P. (1979). Visual evoked responses in rats exposed to heavy metals. In: *Neurotoxicity of the Visual System.* Eds., Weiss, B. and Merigan, W., Raven Press, New York, 203-218.

2. **Fox,D.A.**, Lowndes,H.E. and Bierkamper G.G. (1982). Electrophysiological test methods in neurotoxicology. In: *Target Organ Toxicity The Nervous System.* Ed. Mitchell, C. L., Raven Press, New York, 299-335.

3. **Fox D.A.** (1984). Psychophysically and electrophysiologically determined spatial vision deficits in lead-exposed rats have a cholinergic component. In: *Cellular and Molecular Basis of Neurotoxicity.* Ed. Narahashi, T., Raven Press, New York, 123-140.

4. Dayhaw-Barker P., Forbes D., **Fox D.A.**, Lerman S., McGinness J., Waxler M. and Felter, R. (1986). Drug photoxicity and visual health. In: *Optical Radiation and Visual Health.* Eds. Waxler, M. and Hitchins, V. M., CRC Press, Florida, 147-175.

5. **Fox D.A.** (1990). Retinal damage following developmental lead exposure: A rod selective deficit. In *Neurotoxicity of Heavy Metals.* Ed., Foulkes, E.C., CRC Press: Boca Raton, 171-200.

6. **Fox D.A.** (1992). Visual and auditory system alterations following developmental or adult lead exposure: A critical review. In *Human Lead Exposure.* Ed. Needleman, H.L., CRC Press: Boca Raton, 105-123.

7. Srivastava D. and **Fox D.A.** (1997). Molecular interactions between lead and magnesium ions. In *Mineral and Metal Neurotoxicology* , Eds., Yasui, M., Strong, M. J., Ota, K. and Verity, M. A., CRC Press: Boca Raton, 253-261.

8. **Fox D.A.** (1998). Sensory system alterations following occupational exposure to chemicals, In *Occupational Neurotoxicology* , Eds., Manzo, L. and Costa, L.G., CRC Press: Boca Raton, 169-184.

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10. **Fox D.A.** and Boyes W.K. (2003). Toxic responses of the ocular and visual system, In *Casarett & Doull's Essentials of Toxicology.* 6th Edition, Eds., Klaassen, C.D. and Watkins, J.B., McGraw-Hill Press: New York, 252-265.

11. **Fox D.A.** and Boyes W.K. (2007). Toxic responses of the ocular and visual system, In *Casarett & Doull's Essentials of Toxicology.* 7th Edition. Eds., Klaassen, C.D. and Watkins, J.B., McGraw-Hill Press: New York, pp. 665-697.

## EDITED BOOKS / PROCEEDINGS

1. Costa L.G., Cranmer J.M., **Fox D.A.**, Harry G.J., Reuhl, K.R., Slikker, W., Tilson H.A. and Verity A.M. (1992). Mechanisms of Developmental Neurotoxicology, Tenth International Neurotoxicology Conference, Little Rock, AR, Intox, Press, Little Rock.

2. Reed D.J., deBethizy D., Buhler D., Bus J., Costa L.G., Dybing E., Eaton D., Fantel A., **Fox D.A.**, et al. (1995). Proceedings of the International Congress of Toxicology - VII. Seattle Washington, Elsevier, Amsterdam.

3. Finkelstein, Y., Bowes W.K., **Fox D.A.**, Aschner, M. (2009). Proceedings of the International Neurotoxicology Association, Twelfth Annual Meeting, Jerusalem, Israel. Elsevier, Amsterdam.

## WRITTEN BOOK REVIEWS

November, 1998: Modern Experimental Biochemistry, 3rd edition by Rodney F. Boyer. Addison Wesley Longham Publishing Co.: CA.

## ABSTRACTS: (Over 320 total)

**Updated: August 2009**

AM

## Mcmahon, Carrie

**From:** csacra@easconsultinggroup.com
**Sent:** Monday, October 26, 2009 10:07 AM
**To:** Mcmahon, Carrie
**Subject:** FW: GRN 294 - Translation
**Attachments:** Hashimoto et al 2009 EngTrans.pdf

Dear Carrie,

Attached is the English translation of the paper that you requested. Dr. Soni asked me to extend his apology as he intended to include it with the rest of the documents, but did not.

Best,
Cathryn

**From:** Mcmahon, Carrie [mailto:Carrie.McMahon@fda.hhs.gov]
**Sent:** Monday, October 26, 2009 9:33 AM
**To:** csacra@easconsultinggroup.com
**Subject:** RE: GRN 294

Cathryn -

Could you please forward a copy (English version) of the following reference, cited in your October 21, 2009 response? Thank you.

**Effect of astaxanthin consumption on superoxide scavenging activity in aqueous humor. Journal of the Eye 26: 229-234, 2009**.

Carrie

Carrie McMahon, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review

tel: (301) 436-1202
email: Carrie.McMahon@fda.hhs.gov

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Pages 000362 - 000370 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000371 - 000376 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

AM

**Mcmahon, Carrie**

**From:** esteele@easconsultinggroup.com
**Sent:** Friday, December 18, 2009 2:50 PM
**To:** Mcmahon, Carrie
**Cc:** csacra@easconsultinggroup.com; msoni@easconsultinggroup.com
**Subject:** FW: your GRAS notice: GRN 294
**Importance:** High

Carrie:

Cathryn has gone home for the day. Therefore, I am forwarding the answer to the question you asked about which edition of the FCC we referenced in GRAS Notice #294. It was the 5th edition. We are sorry for the oversight.

Please let us know if you need any additional information.

Thanks!
Ed

Edward A. Steele
President
EAS Consulting Group, LLC
1-877-327-9808 (toll free)
703-684-4408 (local)
703-684-4428 (fax)
www.easconsultinggroup.com

---

**From:** Madhu Soni [mailto:msoni@easconsultinggroup.com]
**Sent:** Friday, December 18, 2009 2:23 PM
**To:** esteele@easconsultinggroup.com
**Subject:** FW: your GRAS notice: GRN 294
**Importance:** High

Per our discussion
Madhu

---

**From:** Madhu Soni [mailto:msoni@easconsultinggroup.com]
**Sent:** Friday, December 18, 2009 2:16 PM
**To:** 'csacra@easconsultinggroup.com'
**Subject:** RE: your GRAS notice: GRN 294
**Importance:** High

Cathryn,
Please send a note to Carrie stating the **Food Chemical Codex Edition should be 5th**. Also express my apology for the oversight. Thanks
Madhu

---

**From:** Cathryn [mailto:csacra@easconsultinggroup.com]
**Sent:** Friday, December 18, 2009 10:33 AM
**To:** msoni@easconsultinggroup.com

**Subject:** FW: your GRAS notice: GRN 294
**Importance:** High

Madhu,

Would you let me know what edition of the FCC you cited?

Thanks,
Cathryn

Cathryn W. Sacra
EAS Consulting Group, LLC
1940 Duke Street
Suite 200
Alexandria, VA 22314
703-684-4438
csacra@easconsultinggroup.com
www.easconsultinggroup.com

**From:** Mcmahon, Carrie [mailto:Carrie.McMahon@fda.hhs.gov]
**Sent:** Friday, December 18, 2009 10:25 AM
**To:** csacra@easconsultinggroup.com
**Subject:** RE: your GRAS notice: GRN 294

Cathryn -

Your notice cites the Food Chemical Codex (FCC) regarding specifications but doesn't provide a reference indicating which edition of FCC. Please confirm which (current (6th) or other edition) you are referring.

Thank you.

Regards,

Carrie McMahon, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review

tel: (301) 436-1202
email: Carrie.McMahon@fda.hhs.gov

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**From:** Cathryn [mailto:csacra@easconsultinggroup.com]
**Sent:** Friday, December 18, 2009 8:42 AM
**To:** Mcmahon, Carrie
**Subject:** RE: your GRAS notice: GRN 294

Dear Carrie,

Many thanks.

Best,

**From:** Mcmahon, Carrie [mailto:Carrie.McMahon@fda.hhs.gov]
**Sent:** Tuesday, December 15, 2009 3:40 PM
**To:** csacra@easconsultinggroup.com
**Subject:** your GRAS notice: GRN 294

Ms. Sacra -

We would like to call you to discuss your GRAS notice for *Haematococcus pluvialis* extract (containing astaxanthin esters) (GRN 294). Would you be available for a telephone call sometime between 9 and 11:30 AM on Thursday, December 17th?

Please let me know.

Regards,

Carrie McMahon, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review

tel: (301) 436-1202
email: Carrie.McMahon@fda.hhs.gov

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AM

**Mcmahon, Carrie**

**From:** esteele@easconsultinggroup.com
**Sent:** Wednesday, December 30, 2009 9:40 AM
**To:** Mcmahon, Carrie
**Cc:** msoni@easconsultinggroup.com; deprince@fujihealthscience.com
**Subject:** Response to Agency Questions - Haematococus pluvialis extract containing astaxanthin esters (GRN 294)
**Attachments:** Carrie McMahon(Fuji Health Science)123009.pdf

Dear Dr. McMahon:
Attached please find our response to the questions raised in our conference call of December 17, 2009 regarding GRAS Notice 294 - *Haematococus pluvialis* extract containing astaxanthin esters.

We appreciate the opportunity to provide this explanation and trust that this satisfactorily addresses the potential concern raised during our discussion. Thank you in advance and we look forward to hearing from you on your conclusion.

Regards,

Edward A. Steele
President
EAS Consulting Group, LLC
1-877-327-9808 (toll free)
703-684-4408 (local)
703-684-4428 (fax)
www.easconsultinggroup.com

Attachment:
Carrie McMahon (Fuji Health Science) 123009

*Specializing in FDA Regulatory Matters*

December 30, 2009

Carrie McMahon, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Division of Biotechnology and GRAS Notice Review
5100 Paint Branch Road
College Park, MD 20740-3835

Dear Dr. McMahon:

**RE: *Haematococus pluvialis* extract containing astaxanthin esters (GRN 294)**

This is in response to the question raised in our conference call on December 17, 2009 regarding our GRAS notice (GRN 000294; *Haematococus pluvialis* extract containing astaxanthin esters). We have addressed the concern about the increased retinal blood flow reported in one study (Nagaki et al., 2005) following ingestion of astaxanthin and its implications to the risk of diabetic retinopathy, particularly during pregnancy. To address this concern, as will be discussed below in more detail, we have taken the following approaches:

1. We will reduce the intended use levels of astaxanthin by 5-fold from what was originally proposed in our Notification;

2. We have critically reviewed the retinal blood flow data reported in Nagaki et al. (2005), and after conducting additional statistical analysis of the data we determined that the differences reported were not statistically significant; and

3. We will discuss the relevance of the two specific studies (Chen et al., 1994; Hellstet et al., 1996) mentioned during the teleconference related to retinal blood flow in pregnant diabetic women.

**1. Reduction of Intended Use Levels**

In the original GRAS submission, Fuji Health Sciences proposed to use astaxanthin esters from *H. pluvialis* at use levels of 0.5 mg astaxanthin/serving in Baked Goods, Beverages, Cereals, Chewing Gum, Coffee and Tea, Dairy Product Analogs, Frozen Dairy Desserts and Mixes, Hard Candy, Milk Products, Processed Fruits and Fruit Juices, Processed Vegetables, Vegetable Juices and Soft Candy. The estimated daily intake of astaxanthin, from the intended uses for the various food categories identified above using CSFII 2003-04, resulted in all users mean and 90$^{th}$ percentile daily exposure of 3.2 and 5.4 mg astaxanthin/person (0.1 mg/kg body weight/day), respectively.

The estimated 90$^{th}$ percentile daily intake of 5.4 mg astaxanthin from the intended uses is similar to the astaxanthin use levels in the study by Nagaki et al. (2005) where increases (9-11%) in retinal capillary

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blood flow in human subjects were reported. As FDA is concerned about this reported effect, Fuji Health Sciences will reduce the intended use levels of 0.5 mg astaxanthin/serving by 5-fold to 0.1 mg/serving. A comparison of the estimated daily intake for different population groups from the earlier use levels and the proposed revised use levels is summarized in the Table below. The revised intended use levels will result in estimated daily all users mean and 90th percentile intake of 0.64 and 1.08 mg astaxanthin/person, respectively. Compared to the dose levels of astaxanthin (6 mg/person) used in Nagaki et al. (2005) study, the revised estimated 90th percentile intake of 1.08 mg astaxanthin/person is 5.5-fold lower and is therefore unlikely to affect retinal blood flow. Moreover, as described below and shown in the Appendices, we determined that the reported increase in retinal blood flow following astaxanthin administration by Nagaki et al. (2005) was not statistically significant.

**Comparison of Revised Estimated Daily Intake of Astaxanthin with Previous Estimates (mg astaxanthin/person/day)**

| Population Group | Previous All-Users Consumption (mg) | | Revised All-Users Consumption (mg) | |
|---|---|---|---|---|
| | Mean | 90th Percentile | Mean | 90th Percentile |
| Infant | 1.1 | 2.3 | 0.22 | 0.46 |
| Child | 2.4 | 3.9 | 0.48 | 0.78 |
| Female Teenager | 2.6 | 4.3 | 0.52 | 0.86 |
| Male Teenager | 3.4 | 5.4 | 0.68 | 1.08 |
| Female Adult | 3.0 | 5.0 | 0.60 | 1.00 |
| Male Adult | 3.8 | 6.3 | 0.76 | 1.26 |
| **Total Population** | **3.2** | **5.4** | **0.64** | **1.08** |

**2. Review of Retinal Blood Flow Study**

In the placebo-controlled study by Nagaki et al. (2005), retinal blood flow and safety were assessed in 18 placebo control and 18 subjects that received daily doses of 6 mg astaxanthin for four-weeks. There were no safety issues. These investigators measured peripapillary blood flow in the superior quadrant of the right and left eyes with a Heidelberg Retinal Flowmeter. The investigators did not provide any technical details on their methods, state which software mode of the instrument was utilized or how/why it was chosen. Based on the retinal blood flow data obtainable with the Heidelberg instrument, it appears that Nagaki et al. (2005) used the automated full-field perfusion image analysis software program. The results of this study reported that there were no significant differences between eyes for either the placebo or the astaxanthin group before ingestion [mean ± SD values were 218 ± 38, 215 ± 28 (left eye), 230 ± 42, 221 ± 32 (right eye) arbitrary units, respectively]. In the before to after ingestion data

comparison, a 2.6-2.8% increase in retinal blood flow in the placebo group and a 9-10.7% increase in retinal blood flow in the astaxanthin group were reported. The results of this study reported that no significant differences between eyes for the placebo group, but a significant difference after astaxanthin ingestion [mean ± SD values were 224 ± 30, 238 ± 34 (left eye), 236 ± 33, 241 ± 35 (right eye) arbitrary units, respectively].

We note that the increase in retinal blood flow in the astaxanthin group was reported as significant at p values of less than 0.01, even though the standard deviations (SDs) were 14-15%. The reported data (mean ± SD) are presented in a pictorial form in Appendix I [also see Appendix II-B for data from Table 2 of Nagaki et al. (2005) in its original form]. The interval data between groups were analyzed with the nonparametric Wilcoxon signed-rank (actually paired-sample) test, which is reserved for ordinal data that does not come from a normal distribution. Moreover, the Wilcoxon test does not correct for repeated measures, which was used in this study. Thus, they did not correctly analyze their data. They should have employed a two-tailed paired-sample t-test or an Analysis of Variance (ANOVA) followed by a multiple post-hoc comparison for repeated measures to analyze this interval data: especially since it appeared to be normally distributed. Based on the original data presented in Table 2 of the Nagaki et al (2005) publication, we were able to calculate p values for the before and after astaxanthin ingestion for each eye using a two-tailed paired-sample t-test. As presented in Appendix II-A, we found that there were no significant differences due to astaxanthin treatment on retinal blood flow. The data from Table 2 of Nagaki et al. (2005) publication in its original form is also presented in Appendix II-B.

**3. Relevance of Studies**

We reviewed the two studies (Chen et al., 1994; Hellstet et al., 1996) mentioned during the teleconference. In both of these studies, changes in retinal blood flow during pregnancy in diabetic women were investigated. In the Chen et al. (1994) study peripapillary retinal venous, <u>not arterial</u>, blood flow was measured in 19 subjects without diabetes and 22 subjects with type I diabetes using laser Doppler velocimetry. Overall, the authors concluded that the autoregulatory capacity was decreased in three to seven of the 22 pregnant diabetic women, depending upon the trimester analyzed. Relative to non-diabetic controls, in trimesters one (n = 3) and two (n = 5) the blood flow was decreased, whereas in trimester three (n = 7) it was increased. It is not clear how the blood velocity and blood flow data were analyzed since the statistics section refers to both the Student's t-test and non-parametric Wilcoxon signed-rank test and no additional information is provided. Regardless, the data analyses are difficult to properly interpret as the authors compared percent changes, ratios of percent changes and the variances ranged from 20-50% of the mean values. Moreover, the SEMs presented in Table 7 of the article overlapped for these small sample sizes (n = 3-7). In summary, the relevance of these results are not clear since venous blood flow was measured, a small sample size was used and the statistical significance of the data is unclear.

In the Hellstedt et al. (1996) study, peripapillary retinal arterial blood flow was measured in 10 subjects without diabetes and 17 subjects with type I diabetes using blue-field entopic simulation. Overall, the authors found that the retinal blood capillary flow was decreased in pregnant diabetic women and thus the autoregulatory capacity was altered. However, the investigators concluded that "pregnancy-induced hyperdynamic circulation and consequent shear stress in macular capillaries is unlikely to contribute to the progression of minimal background retinopathy occurring during pregnancy". The data were analyzed with multiple ANOVA and the results appear valid.

Overall, the results from these and other retinal blood flow studies in diabetic women during pregnancy and the postpartum period are equivocal. For most pregnant diabetic women, the progression of diabetic retinopathy occurs temporarily during pregnancy and postpartum, and it has no long-term detrimental effects unless it progressed to late stage 4 or 5 (Temple et al. 2001; Kaaja and Loukovaara 2007; Sheth 2008). Furthermore, given the reduction in intended use levels of astaxanthin in different food categories that will result in daily exposure of 1.08 mg astaxanthin/person and as the appropriate statistical analysis did not reveal statistically significant differences in retinal blood flow following astaxanthin ingestion in the Nagaki et al (2005) study, we believe that there is no concern related to the retinal blood flow following ingestion of astaxanthin.

**Conclusion**

To address FDA's potential safety concern related to the effects of astaxanthin on retinal blood flow in individuals with diabetic retinopathy, the intended use levels of astaxanthin will be reduced 5-fold. The new intended use levels will result in 90$^{th}$ percentile astaxanthin intake of 1.08 mg/person/day. Reevaluations of the data (mean ± SD values) and application of a two-tailed paired-sample t-test for analysis of the Nagaki et al. (2005) data indicate that the appropriate statistical methods were not used and the statistical significance reported in the study was not correct. Additionally, the results from publicly available retinal blood flow studies in diabetic women during pregnancy and the postpartum period are equivocal. We conclude that the planned reduction in the proposed use levels of astaxanthin in the specified foods in our Notification by 5-fold is unlikely to cause any adverse effects in subjects with diabetes/diabetic retinopathy.

We appreciate the opportunity to provide this explanation and our additional analyses of the aforementioned studies and trust that this satisfactorily addresses the potential concern raised during our discussion of December 17, 2009. Thank you in advance and we look forward to hearing from you on your conclusion.

Best regards

Edward A. Steele
President

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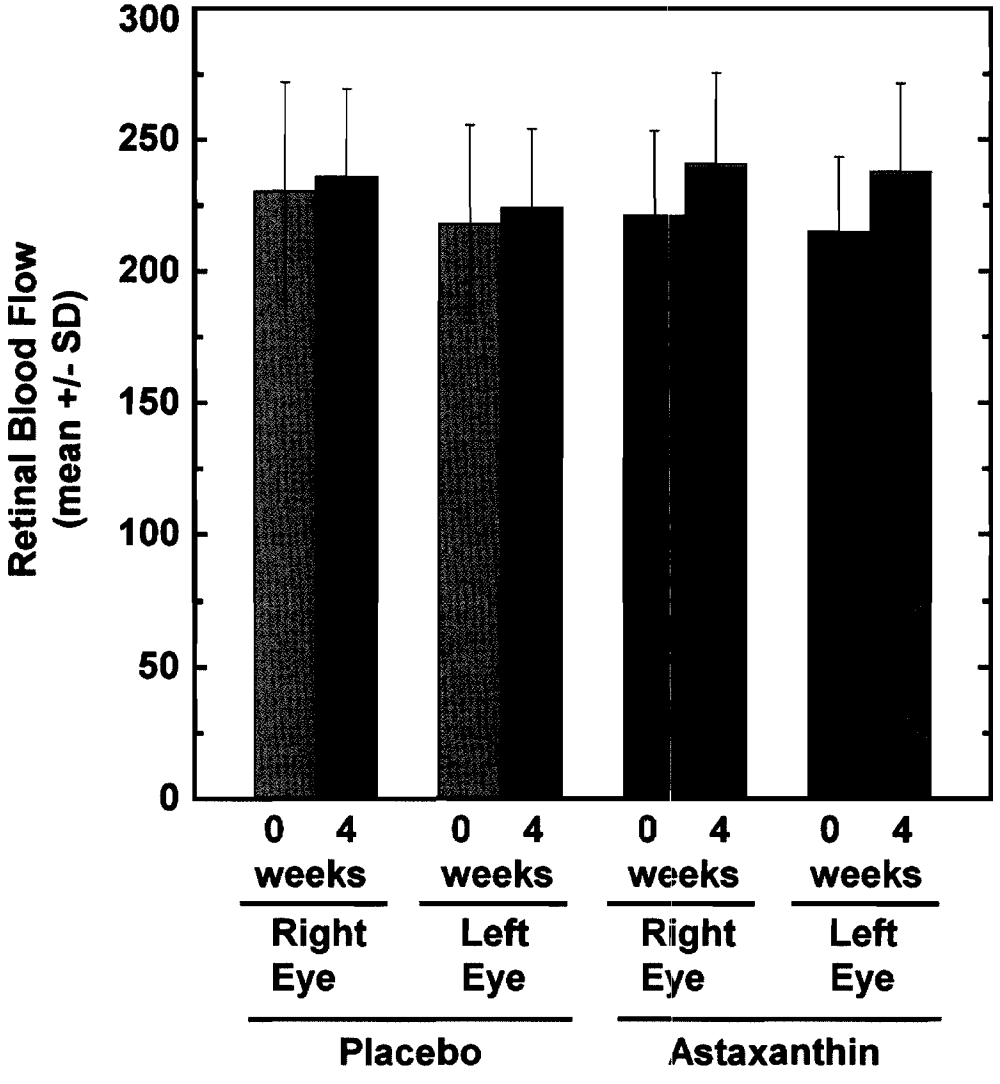
**Appendix I**

**Retinal capillary blood flow data (mean ± SD) (Nagaki et al., 2005) (also see Appendix II-B).**




**Data from Nagaki et al. J Clin Therap Med 21: 537-542, 2005.**

**Appendix II-A**

**Two-tailed paired-sample t-test for the Nagaki et al. (2005) data**

The two-tailed paired sample t test was used to reanalyze the data from Table 2 of Nagaki et al. (2005). This analysis utilizes the sample size, difference between means and the standard deviation to calculate a t value and subsequently a p value in a look-up table with Critical Values of the t Distribution (Zar, 1974 and Table D.10 therein). The comparisons reveal the following: A) placebo right eye after ingestion vs before ingestion $t_{0.05(2),17} = 0.23$, $p>0.50$, B) placebo left eye after ingestion vs before ingestion $t_{0.05(2),17} = 0.25$, $p>0.50$, C) astaxanthin right eye after ingestion vs before ingestion $t_{0.05(2),17} = 0.84$, $p>0.25$, and D) astaxanthin left eye after ingestion vs before ingestion $t_{0.05(2),17} = 01.05$, $p>0.25$. As seen in Appendix I, the SDs overlap for all groups. In summary, the above statistical analysis reveals that astaxanthin did not significantly increase retinal blood flow following administration of 6 mg astaxanthin/day for 4 weeks.

Zar, J.H. 1974. Paired-sample hypothesis. In: Biostatistical Analysis, Prentice-Hall, Engelwood Cliffs, NJ, pp. 121-129 and Table D.10 on p. 413.

**Appendix II-B**

Data from Table 2 of Nagaki et al. (2005) in its original form.

**Table 2 Retinal capillary perfusion (Flow)**

| | | AX group | Placebo group |
|---|---|---|---|
| Before ingestion | Right eye | 221±32 | 230±42 |
| | Left eye | 215±28 | 218±38 |
| After ingestion | Right eye | 241±35[a] (9.0%[b]) | 236±33 (2.6%) |
| | Left eye | 238±34[a] (10.7%[b]) | 224±30 (2.8%) |

a: $p<0.01$ (comparison before and after ingestion), b: $p<0.01$ (AX group vs. placebo group)

(%) Percent of increase in value after ingestion

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