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AIBMR Life Sciences, Inc.

October 11, 2018

Susan Carlson, PhD
Division Director
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Department of Health and Human Services
5001 Campus Drive
College Park, MD 20740

Dear Dr. Carlson:

In accordance with regulation 21 CFR Part 170 Subpart E (Generally Recognized as Safe (GRAS) Notice), on behalf of Wiley Organics, Inc., d/b/a Organic Technologies (the notifier), the undersigned, Timothy S. Murbach, submits, for FDA review, the enclosed notice that AlaskOmega® Omega-7 500 & 700 (C16:1 fish oils) are GRAS for use in foods.

Should you have any questions or concerns regarding this notice, please contact me at 253-286-2888 or tim@aibmr.com.

Sincerely,

[redacted]

Timothy S. Murbach, ND, DABT (agent of the notifier)
Senior Scientific & Regulatory Consultant
AIBMR Life Sciences, Inc. ("AIBMR")

# 819

# Notice to US Food and Drug Administration of the Conclusion that the Intended Uses of AlaskOmega® Omega-7 500 & 700 are Generally Recognized as Safe

**Submitted by the Notifier:**

Wiley Organics, Inc.
d/b/a Organic Technologies

**Prepared by the Agent of the Notifier:**

AIBMR Life Sciences, Inc.
2800 E. Madison, Suite 202
Seattle WA 98112

**October 11, 2018**

# Table of Contents

# Part 1: Signed Statements and Certification

## 1.1 Submission of GRAS Notice

Wiley Organics, Inc., d/b/a Organic Technologies (the notifier) is submitting a new GRAS notice in accordance with Title 21 of the U.S. Code of Federal Regulations (CFR), Chapter I, Subchapter B, Part 170, Subpart E, regarding the conclusion that AlaskOmega® Omega-7 500 and 700 (C16:1 fish oils) are Generally Recognized as Safe (GRAS) for their intended use, consistent with section 201(s) of the Federal Food, Drug and Cosmetic Act.

## 1.2 Name and Address of the Notifier and Agent of the Notifier

### Notifier

Steve Dillingham
Global Director, AlaskOmega Ingredients
Wiley Organics, Inc., d/b/a Organic Technologies
545 Walnut Street
Coshocton, Ohio 43812
Tel: (740) 622-0755; Fax: (740) 622-3231
sales@alaskomega.com

### Agent of the Notifier

Timothy S. Murbach, ND, DABT
Senior Scientific & Regulatory Consultant
AIBMR Life Sciences, Inc.
2800 E. Madison
Seattle, WA 98112
Tel: (253) 286-2888
tim@aibmr.com

## 1.3 Name of the Substance

AlaskOmega® Omega-7 500

AlaskOmega® Omega-7 700

The ingredients are mixtures of concentrated edible fatty acid ethyl esters derived from crude Alaska pollock (*Gadus chalcogrammus,* Pallas, 1814) oil. Omega-7 500 contains at least 500 mg/g palmitoleic acid (C16:1, *cis*-$\Delta^9$) ethyl ester. Omega-7 700 contains at least 700 mg/g palmitoleic acid ethyl ester.

## 1.4 Intended Conditions of Use

AlaskOmega® Omega-7 500 and 700 are intended to be used as nutritive ingredients in food to replace other edible fats and oils.

AlaskOmega® Omega-7 500 and 700 are not intended for use in foods where standards of identity would preclude such use, infant formula, meat, poultry, egg products, catfish, or any products that would require additional regulatory review by USDA.

## 1.5 Statutory Basis for GRAS Conclusion

The conclusion of GRAS status of AlaskOmega® Omega-7 500 and 700 for their intended conditions of use, stated in Part 1.4 of this report, has been made based on scientific procedures.

## 1.6 Not Subject to Premarket approval

We have concluded that AlaskOmega® Omega-7 500 and 700 are GRAS for their intended conditions of use, stated in Part 1.4 of this report, and, therefore, such use is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

## 1.7 Data and Information Availability Statement

The data and information that serve as the basis for this GRAS conclusion will be available for review and copying during customary business hours at the office of Michael Leman, 545 Walnut Street, Coshocton, OH 43812, or will be sent to FDA upon request.

## 1.8 Exemption from Disclosure under the Freedom of Information Act

None of the data and information in Parts 2 through 7 of this GRAS notice are considered exempt from disclosure under the Freedom of Information Act (FOIA)

as trade secret or commercial or financial information that is privileged or confidential.

## 1.9 Certification of Completion

We hereby certify that, to the best of our knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of AlaskOmega® Omega-7 500 and 700.

October 11, 2018

Steve Dillingham
Global Director, AlaskOmega Ingredients
Wiley Organics, Inc., d/b/a Organic Technologies

Date

# Part 2: Identity, Manufacture, Specifications, and Physical or Technical Effect

## 2.1 Identification

AlaskOmega® Omega-7 500 and 700 are highly refined, molecularly distilled, purified mixtures of edible fatty acid esters derived from crude Alaska pollock oil. Alaska pollock (*Gadus chalcogrammus*, Pallas, 1814; synonym *Theragra chalcogramma* (Pallas, 1814); also known by common name walleye pollock) is a member of the cod family, Gadidae, is widely distributed in the temperate to boreal North Pacific (FAO Area 67),[1] and has been among the world's most harvested fish, topping the most recent list in 2014.[2]

The predominant fatty acid in AlaskOmega® Omega-7 ingredients is palmitoleic acid (C16:1, *cis*-$\Delta^9$, IUPAC name (9*Z*)-Hexadec-9-enoic acid, CAS RN 373-49-9, synonym *cis*-9-hexadecenoic acid), a monounsaturated omega-7 fatty acid with a chemical formula of $C_{16}H_{30}O_2$ and molar mass of 254.41 g/mol. The structure of palmitoleic acid is shown in Figure 1. AlaskOmega® Omega-7 500 and 700 are concentrated to provide a minimum of 500 mg/g (50%) or 700 mg/g (70%) palmitoleic acid, respectively. Typical certificates of analyses indicate that actual concentrations of palmitoleic acid are within a range of +4% of the minimum specification (see Tables 4 & 5).

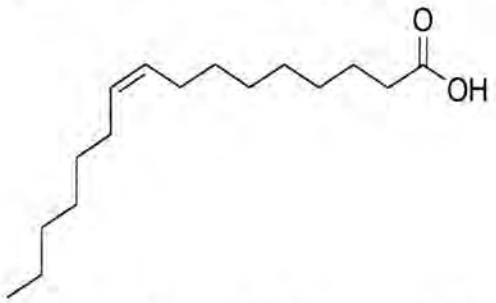


**Figure 1.** Chemical Structure of Palmitoleic Acid

The second most prominent fatty acid present in AlaskOmega® Omega-7 500 and 700 is the saturated precursor of palmitoleic acid, palmitic acid (C16:0, IUPAC name hexadecanoic acid). Palmitic acid has the chemical formula $C_{16}H_{32}O_2$ and a molar mass of 256.43 g/mol. AlaskOmega® Omega-7 500 and 700 provide a minimum of 40 mg/g (4%) or 50 mg/g (5%) palmitic acid, respectively, but more typically contain 10–20% palmitic acid.

AlaskOmega® Omega-7 500 and 700 have little to no eicosapentanoic (EPA) or docosahexaenoic (DHA) acid ($\leq$ 0.2% each) and little to no *trans* fatty acids.

AlaskOmega® Omega-7 ingredients do not contain industrially produced semi-synthetic *trans* fatty acids, and typical fatty acid profiles demonstrate that <1% of naturally occurring *trans* fatty acids are present. Other minor fatty acids present include saturated, monounsaturated, and polyunsaturated fatty acids typical of edible fish oils. With the exception of oleic acid, which may be present in low single digit percentages, most other fatty acids are present at <1% each. The fatty acid percentages may vary slightly; however, the palmitoleic acid, palmitic acid, EPA, and DHA concentrations are controlled (see Specification Tables 2 and 3). Typical fatty acid profiles are presented in Table 1 below (note, the percent values shown in the table below (as well as Table 8) were converted from measured values in mg/g).

**Table 1:** Typical Fatty Acid Profiles of AlaskOmega® Omega-7 Products

| Fatty Acid Ethyl Esters | | AlaskOmega® Omega-7 500 (% w/w) | AlaskOmega® Omega-7 700 (% w/w) |
|---|---|---|---|
| Common Name | Lipid numbers + $\Delta^X$ | | |
| Myristic | C14:0 | 0.08 | 0.02 |
| Myristelaidic | C14:1, *trans*-$\Delta^9$ | 0.02 | 0.00 |
| Myristoleic | C14:1, *cis*-$\Delta^9$ | 0.48 | 0.03 |
| Palmitic | C16:0 | 20.35 | 10.44 |
| Palmitoleic | C16:1, *cis*-$\Delta^9$ | 50.52 | 71.98 |
| Stearic | C18:0 | 0.11 | 0.00 |
| *trans*-octadecenoic | C18:1, *trans*-(isomer unidentified) | 0.93 | 0.00 |
| Oleic | C18:1, *cis*-$\Delta^9$ | 5.31 | 0.00 |
| *cis*-Vaccenic | C18:1, *cis*-$\Delta^{11}$ | 2.01 | 0.00 |
| Linoleic | C18:2, all-*cis*-$\Delta^{9,12}$ | 0.56 | 0.00 |
| Gamma-linolenic | C18:3, all-*cis*-$\Delta^{6,9,12}$ | 0.06 | 0.00 |
| Alpha-linolenic | C18:3, all-*cis*-$\Delta^{9,12,15}$ | 0.31 | 0.00 |
| Stearidonic | C14:4, all-*cis*-$\Delta^{6,9,12,15}$ | 0.77 | 0.00 |
| Gadoleic | C20:1, *cis*-$\Delta^9$ | 0.04 | 0.00 |
| Gondoic | C20:1, *cis*-$\Delta^{11}$ | 0.02 | 0.00 |
| EPA | C20:5, all-*cis*-$\Delta^{5,8,11,14,17}$ | 0.11 | 0.07 |
| Docosanoic | C22:0 | 0.04 | 0.00 |
| Docosapentaenoic | C22:5, all-*cis*-$\Delta^{7,10,13,16,19}$ | 0.03 | 0.00 |
| DHA | C22:6, all-*cis*- $\Delta^{4,7,10,13,16,19}$ | 0.20 | 0.02 |
| Unidentified C16 & C18 polyunsaturated ethyl esters | | 16.62 | 15.13 |

AlaskOmega® Omega-7 500 and 700 contain ethyl ester forms of fatty acids isolated from Alaska pollock oil. Fats and oils from natural sources are predominantly found in the triglyceride form. Triglycerides, or more formally, triacylglycerols, found in both animal and plant-derived fats and oils, are comprised of three fatty acids (which can be saturated, monounsaturated, or polyunsaturated) esterified to a glycerol backbone. Fatty acid ethyl esters, on the other hand, are formed when fatty acids are

*trans*-esterified whereby the glycerol backbone of a triglyceride is removed and substituted with ethanol (see Manufacturing Overview).

## 2.2 Manufacturing

### 2.2.1 Manufacturing Overview

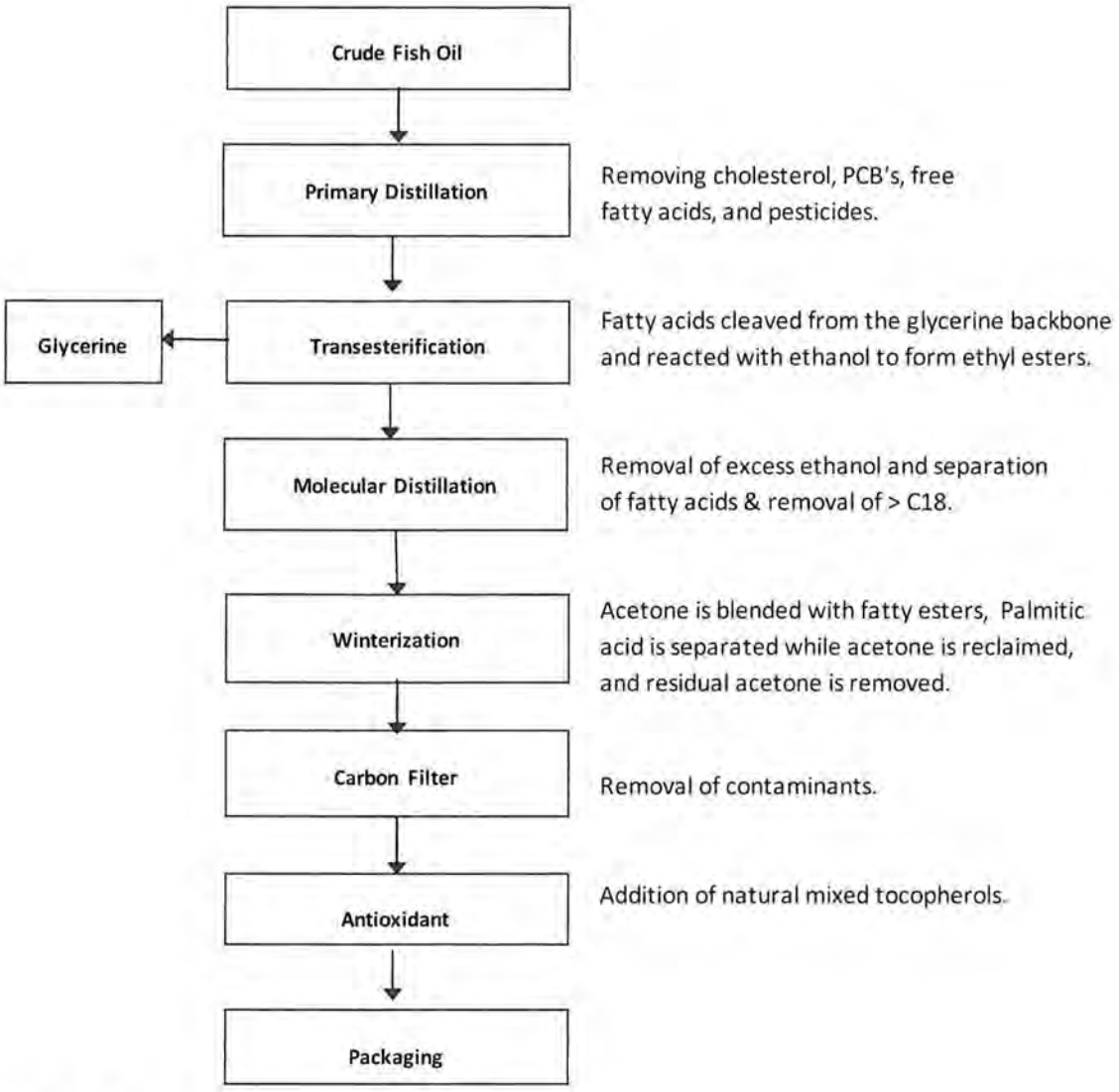


**Figure 2.** Manufacturing Flowchart

**Distillation:** The crude fish oil is heated under high vacuum to remove polychlorinated biphenyls (PCB's), free fatty acids, and pesticides.

**Transesterification:** Ethanol and the heat-treated crude fish oil are combined into a reaction vessel. Sodium ethoxide in ethanol is added and the reaction mixture is heated. Once the reaction is determined to be complete, the reaction is cooled and allowed to settle. The bottom glycerol layer is then separated.

**Molecular Distillation (in 2 steps):** (1) The excess ethanol is removed via distillation. (2) The fatty acid ethyl esters (FAEE's) are then distilled under vacuum to remove the higher boiling FAEE's (>C18).

**Winterization:** The saturated and unsaturated fatty acid ethyl esters (C16's) are blended with acetone and chilled to free saturated fatty acid. The resulting slurry is filtered, and the acetone is then removed via distillation to leave an enriched C16 monounsaturated fatty acid concentrate.

**Carbon Filtration:** Contaminants are removed by passing through a carbon filter.

**Addition of Mixed Tocopherols:** Natural mixed tocopherols are added as an antioxidant.

**Filling:** The product is packaged into 55-gallon closed head steel drums.

### 2.2.2 Good Manufacturing Practice

AlaskOmega® Omega-7 500 and 700 from Organic Technologies are produced in an FDA registered facility under strict adherence to NSF/ANSI Standard 173, Section 8 current GMP standards set to comply with the U.S. Code of Federal Regulations, 21 CFR Part 111 and certified by NSF International.

### 2.2.3 Raw Materials

All raw materials used in the production of Organic Technologies' AlaskOmega® Omega-7 500 and 700 are obtained from qualified suppliers according to standard operating procedure. Crude oil used in the production of Organic Technologies' AlaskOmega® Omega-7 500 and 700 is derived from wild-caught Alaska pollock (*Gadus chalcogrammus*) harvested from the North Pacific and the Alaskan Bering Sea fisheries (FAO Area 67), which are managed by the National Oceanic and Atmospheric Administration's National Marine Fisheries Services, a division of the U.S. Department of Commerce. All raw materials used are suitable for use in production of a food grade ingredient; ethanol, acetone, and natural mixed tocopherols conform to US Pharmacopeia requirements. No materials of human origin or derived from, or exposed to, animals affected by, or under quarantine for transmitting, Animal Spongiform Encephalopathy/ Bovine Spongiform

Encephalopathy are used. AlaskOmega® Omega-7 500 and 700 from Organic Technologies are non-GMO and not irradiated.

## 2.3 Specifications

The specifications for the food-grade products AlaskOmega® Omega-7 500 and 700, along with the specification methods, are listed in Tables 2 and 3 below.

**Table 2.** AlaskOmega® Omega-7 500 Specifications

| Test Parameters | Specification | Method |
|---|---|---|
| **Marker Compounds** | | |
| Palmitoleic acid (as EE) | NLT 500 mg/g | QC-193C (GC-FID) |
| Palmitic acid (as EE) | NLT 40 mg/g | QC-193C (GC-FID) |
| Palmitoleic:Palmitic Ratio | NMT 15:1 | Calculated |
| Eicosapentaenoic acid (as EE) | NMT 2 mg/g | QC-193C (GC-FID) |
| Docosahexaenoic acid (as EE) | NMT 2 mg/g | QC-193C (GC-FID) |
| **Physical Characteristics** | | |
| Appearance | Bright & clear, colorless to pale yellow oily liquid | QC-529 |
| Color | NMT 4 Gardner | QC-536 |
| **Impurities** | | |
| Acid value | NMT 1.0 mg KOH/g | AOCS Cd 3d-63 |
| Anisidine value | NMT 5 | AOCS Cd 18-90 |
| Peroxide value | NMT 1.0 meq/kg | AOCS Cd 8b-90 |
| Total Oxidation, TOTOX (2 x Peroxide + anisidine) | NMT 5 | Calculated |
| Cholesterol | NMT 0.1% | QC-186M |
| Oligomers & partial glycerides | NMT 1.0% | QC-816 |
| **Organic Contaminants** | | |
| PCBs (209 Congeners) | NMT 0.045 mg/kg | EPA 1668A |
| PCDDs & PCDFs | NMT 1 pg WHO-PCDD/F-TEQ/g | EPA 1613B |
| Dioxin-like PCBs | NMT 2 pg WHO-TEQ/g | EPA 1668A |
| Total Dioxins, Furans, & Dioxin-like PCBs | NMT 2 pg WHO-TEQ/g | EPA 1668A/1613B |
| **Heavy Metals** | | |
| Arsenic | NMT 0.1 mg/kg | AOAC 993.14 (ICP-MS) |
| Cadmium | NMT 0.01 mg/kg | AOAC 993.14 (ICP-MS) |
| Lead | NMT 0.01 mg/kg | AOAC 993.14 (ICP-MS) |
| Mercury | NMT 0.01 mg/kg | AOAC 993.14 (ICP-MS) |
| **Microbiological Tests** | | |
| Total Aerobic Microbial | <10 CFU/g | USP <61> |
| Yeast | <10 CFU/g | USP <61> |
| Mold | <10 CFU/g | USP <61> |

| | | |
|---|---|---|
| *E. coli* | Negative/10 g | USP <62> |
| *Salmonella* | Negative/10 g | USP <62> |
| *Staphylococcus* | Negative/10 g | USP <62> |
| **Residual Solvents** | | |
| Acetone | NMT 30 ppm | USP <467> |

Abbreviations: AOCS, American Oil Chemists Society; CFU, colony forming units; EE, ethyl ester; EPA, United States Environmental Protection Agency; GC-FID, gas chromatography with flame ionization detector; ICP-MS, inductively coupled plasma-mass spectrometry; KOH, potassium hydroxide; NLT, not less than; NMT, not more than; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzodioxins; PCDF, polychlorinated dibenzofurans; QC, internal method; TEQ, toxic equivalency; USP, United States Pharmacopeia; WHO, World Health Organization.

## **Table 3.** AlaskOmega® Omega-7 700 Specifications

| Test Parameters | Specification | Method |
|---|---|---|
| **Marker Compounds** | | |
| Palmitoleic acid (as EE) | NLT 700 mg/g | QC-193C (GC-FID) |
| Palmitic acid (as EE) | NLT 50 mg/g | QC-193C (GC-FID) |
| Palmitoleic:Palmitic Ratio | NMT 18:1 | Calculated |
| Eicosapentaenoic acid (as EE) | NMT 2 mg/g | QC-193C (GC-FID) |
| Docosahexaenoic acid (as EE) | NMT 2 mg/g | QC-193C (GC-FID) |
| **Physical Characteristics** | | |
| Appearance | Bright & clear, colorless to pale yellow oily liquid | QC-529 |
| Color | NMT 4 Gardner | QC-536 |
| **Impurities** | | |
| Acid value | NMT 1.0 mg/KOH/g | AOCS Cd 3d-63 |
| Anisidine value | NMT 5 | AOCS Cd 18-90 |
| Peroxide value | NMT 1.0 meq/kg | AOCS Cd 8b-90 |
| Total Oxidation, TOTOX (2 x Peroxide + anisidine) | NMT 5 | Calculated |
| Cholesterol | NMT 0.1% | QC-186M |
| Oligomers & partial glycerides | NMT 1.0% | QC-816 |
| **Organic Contaminants** | | |
| PCBs (209 Congeners) | NMT 0.045 mg/kg | EPA 1668A |
| PCDDs & PCDFs | NMT 1 pg WHO-PCDD/F-TEQ/g | EPA 1613B |
| Dioxin-like PCBs | NMT 2 pg WHO-TEQ/g | EPA 1668A |
| Total Dioxins, Furans, & Dioxin-like PCBs | NMT 2 pg WHO-TEQ/g | EPA 1668A/1613B |
| **Heavy Metals** | | |
| Arsenic | NMT 0.1 mg/kg | AOAC 993.14 (ICP-MS) |
| Cadmium | NMT 0.01 mg/kg | AOAC 993.14 (ICP-MS) |
| Lead | NMT 0.01 mg/kg | AOAC 993.14 (ICP-MS) |
| Mercury | NMT 0.01 mg/kg | AOAC 993.14 (ICP-MS) |
| **Microbiological Tests** | | |
| Total Aerobic Microbial | <10 CFU/g | USP <61> |
| Yeast | <10 CFU/g | USP <61> |
| Mold | <10 CFU/g | USP <61> |

| | | |
|---|---|---|
| *E. coli* | Negative/10 g | USP <62> |
| *Salmonella* | Negative/10 g | USP <62> |
| *Staphylococcus* | Negative/10 g | USP <62> |
| **Residual Solvents** | | |
| Acetone | NMT 30 ppm | UPS <467> |

Abbreviations: AOCS, American Oil Chemists Society; CFU, colony forming units; EE, ethyl ester; EPA, United States Environmental Protection Agency; GC-FID, gas chromatography with flame ionization detector; ICP-MS, inductively coupled plasma-mass spectrometry; KOH, potassium hydroxide; NLT, not less than; NMT, not more than; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzodioxins; PCDF, polychlorinated dibenzofurans; QC, internal method; TEQ, toxic equivalency; USP, United States Pharmacopeia; WHO, World Health Organization.

### 2.3.1 Batch Analysis

Organic Technologies' AlaskOmega® Omega-7 500 and 700 are tested in production lots. Batch analyses of three non-consecutive lots of Omega-7 500 and two non-consecutive lots of Omega-7 700, representing approximately 21 and 10 months of production of the products, respectively, are shown below and were reasonably consistent and met the product specifications.

**Table 4.** AlaskOmega® Omega-7 500 Batch Analyses

| Test Parameters | Specification | Lot No./Data of Manufacture | | |
|---|---|---|---|---|
| | | [redacted] 01/27/2017 | [redacted] 10/20/2017 | [redacted] 09/13/2018 |
| **Marker Compounds** | | | | |
| Palmitoleic acid (as EE) | NLT 500 mg/g | 537 | 515 | 521 |
| Palmitic acid (as EE) | NLT 40 mg/g | 223 | 146 | 78 |
| Palmitoleic:Palmitic Ratio | NMT 15:1 | 2.4:1 | 3.5:1 | 6.7:1 |
| Eicosapentaenoic acid (as EE) | NMT 2 mg/g | 0.5 | 0.3 | 0.7 |
| Docosahexaenoic acid (as EE) | NMT 2 mg/g | 0.6 | 0.3 | 0.2 |
| **Physical Characteristics** | | | | |
| Appearance | Bright & clear, colorless to pale yellow oily liquid | Complies | Complies | Complies |
| Color | NMT 4 Gardner | 2+ | 2 | 2 |
| **Impurities** | | | | |
| Acid value | NMT 1.0 mg KOH/g | 0.1 | 0.3 | 0.2 |
| Anisidine value | NMT 5 | <0.1 | 2.1 | 3.7 |
| Peroxide value | NMT 1.0 meq/kg | <0.1 | <0.1 | 0.6 |
| Total Oxidation, TOTOX (2 x Peroxide + anisidine) | NMT 5 | <0.1 | 2 | 4.9 |
| Cholesterol | NMT 0.1% | <0.01 | <0.01 | <0.01 |
| Oligomers & partial glycerides | NMT 1.0% | 0.6 | 0.6 | 0.6 |
| **Organic Contaminants** | | | | |
| PCBs (209 Congeners) | NMT 0.045 mg/kg | <0.01 | <0.01 | <0.01 |
| PCDDs & PCDFs | NMT 1 pg WHO-PCDD/F-TEQ/g | 0.24 | 0.12 | 0.11 |

| | | | | |
|---|---|---|---|---|
| Dioxin-like PCBs | NMT 2 pg WHO-TEQ/g | 0.45 | 0.30 | 1.00 |
| Total Dioxins, Furans, & Dioxin-like PCBs | NMT 2 pg WHO-TEQ/g | 0.69 | 0.42 | 1.11 |
| **Heavy Metals** | | | | |
| Arsenic | NMT 0.1 mg/kg | 0.017 | <0.007 | 0.003 |
| Cadmium | NMT 0.01 mg/kg | <0.002 | <0.002 | <0.002 |
| Lead | NMT 0.01 mg/kg | <0.006 | <0.006 | <0.002 |
| Mercury | NMT 0.01 mg/kg | <0.003 | <0.003 | <0.002 |
| **Microbiological Tests** | | | | |
| Total Aerobic Microbial | <10 CFU/g | <10 | <10 | <10 |
| Yeast | <10 CFU/g | <10 | <10 | <10 |
| Mold | <10 CFU/g | <10 | <10 | <10 |
| *E. coli* | Negative/10 g | Negative | Negative | Negative |
| *Salmonella* | Negative/10 g | Negative | Negative | Negative |
| *Staphylococcus* | Negative/10 g | Negative | Negative | Negative |
| **Residual Solvents** | | | | |
| Acetone (current spec)* | NMT 30 ppm | N/A | N/A | 1.31 |
| Methanol+Heptane (old spec)* | NMT 30 ppm total | Pass | Pass | N/A |

Abbreviations: CFU, colony forming units; EE, ethyl ester; KOH, potassium hydroxide; N/A, not applicable; NLT, not less than; NMT, not more than; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzodioxins; PCDF, polychlorinated dibenzofurans; TEQ, toxic equivalency; WHO, World Health Organization.
*See Subpart 2.3.2 below.

## Table 5. AlaskOmega® Omega-7 700 Batch Analyses

| Test Parameters | Specification | Lot No./Data of Manufacture | |
|---|---|---|---|
| | | [redacted] 01/28/2017 | [redacted] 11/08/2017 |
| **Marker Compounds** | | | |
| Palmitoleic acid (as EE) | NLT 700 mg/g | 700 | 720 |
| Palmitic acid (as EE) | NLT 50 mg/g | 81 | 104 |
| Palmitoleic:Palmitic Ratio | NMT 18:1 | 8.7:1 | 6.9:1 |
| Eicosapentaenoic acid (as EE) | NMT 2 mg/g | 0.2 | 0.7 |
| Docosahexaenoic acid (as EE) | NMT 2 mg/g | 0.2 | 0.2 |
| **Physical Characteristics** | | | |
| Appearance | Bright & clear, colorless to pale yellow oily liquid | Complies | Complies |
| Color | NMT 4 Gardner | 2+ | 2 |
| **Impurities** | | | |
| Acid value | NMT 1.0 mg/KOH/g | 0.1 | 0.1 |
| Anisidine value | NMT 5 | 1.7 | <0.1 |
| Peroxide value | NMT 1.0 meq/kg | <0.1 | <0.1 |
| Total Oxidation, TOTOX (2 x Peroxide + anisidine) | NMT 5 | 2 | <0.1 |
| Cholesterol | NMT 0.1% | <0.01 | 0.02 |
| Oligomers & partial glycerides | NMT 1.0% | 0.2 | 0.6 |
| **Organic Contaminants** | | | |

| | | | |
|---|---|---|---|
| PCBs (209 Congeners) | NMT 0.045 mg/kg | <0.01 | <0.01 |
| PCDDs & PCDFs | NMT 1 pg WHO-PCDD/F-TEQ/g | 0.26 | 0.12 |
| Dioxin-like PCBs | NMT 2 pg WHO-TEQ/g | 0.49 | 0.81 |
| Total Dioxins, Furans, & Dioxin-like PCBs | NMT 2 pg WHO-TEQ/g | 0.75 | 0.93 |
| **Heavy Metals** | | | |
| Arsenic | NMT 0.1 mg/kg | 0.021 | <0.005 |
| Cadmium | NMT 0.01 mg/kg | <0.002 | <0.001 |
| Lead | NMT 0.01 mg/kg | <0.005 | <0.005 |
| Mercury | NMT 0.01 mg/kg | <0.003 | <0.002 |
| **Microbiological Tests** | | | |
| Total Aerobic Microbial | <10 CFU/g | <10 | <10 |
| Yeast | <10 CFU/g | <10 | <10 |
| Mold | <10 CFU/g | <10 | <10 |
| *E. coli* | Negative/10 g | Negative | Negative |
| *Salmonella* | Negative/10 g | Negative | Negative |
| *Staphylococcus* | Negative/10 g | Negative | Negative |
| **Residual Solvents** | | | |
| Acetone (current spec)* | NMT 30 ppm | N/A | N/A |
| Methanol+Heptane (old spec)* | NMT 30 ppm total | Pass | Pass |

Abbreviations: CFU, colony forming units; EE, ethyl ester; KOH, potassium hydroxide; N/A, not applicable; NLT, not less than; NMT, not more than; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzodioxins; PCDF, polychlorinated dibenzofurans; TEQ, toxic equivalency; WHO, World Health Organization.
*See Subpart 2.3.2 below.

### 2.3.2 Residual Solvent Analysis

Finished product specifications are set to ensure that production controls maintain residual solvent levels below the accepted and established guidelines set forth by FDA and the International Conference for Harmonization (ICH) ICH Q3C. Solvents used in the production of AlaskOmega® Omega-7 500 and 700 are ethanol (class 3) and acetone (class 3). Use of these solvents are recent manufacturing changes to the manufacturing process and product specifications that were effective as of September 13, 2018. The previously used solvents were methanol (class 2) and heptane (class 3). One batch analysis for the AlaskOmega® Omega-7 500 ingredient produced after the effective date was provided in order to demonstrate the ability to comply with the new specification.

### 2.3.3 Residual Pesticide Analysis

In accordance with standard operating procedures, Organic Technologies is committed to 3rd party skip-lot testing of AlaskOmega® Omega-7 500 and 700 for pesticide residues. Although no pesticides are used in the production of AlaskOmega® Omega-7 ingredients, due to ocean pollution concerns, lots are periodically analyzed for the presence of organochlorine, pyrethroid,

organonitrogen, and organophosphate residual pesticides by an independent laboratory. A representative analytical report for AlaskOmega® Omega-7 500 lot number [redacted] and AlaskOmega® Omega-7 700 lot number [redacted] was reviewed by the Panel and demonstrated the lots were free of all pesticides tested at the reporting limits

### 2.3.4 Shelf–Life Stability

A three-year shelf–life from the time of manufacture has been recommended as an appropriate expiration period for AlaskOmega® Omega-7 500. This recommendation is based upon test results of a 36-month long-term stability test of production lot number [redacted] (manufactured December 16, 2014). The long-term stability test was conducted under conditions of commercial storage at 20–30 °C, (as standard operating procedure for storage of AlaskOmega® Omega-7 500 does not require humidity control, this was not part of the monitoring protocol) and commercial packaging (under nitrogen in a lined 55-gallon steel closed head drum with a 3-inch bung seal). Each 8-ounce sample for analysis was taken under argon purge in order to insure minimal potential for oxygen exposure, and peroxide and anisidine tests were performed first. At all time points, outcome measures included the same physical and chemical tests and test methodologies used for commercial batch analysis. The measures were stable and within specification throughout the test with no significant changes occurring in the parameters assayed.

## 2.4 Physical or Technical Effect

AlaskOmega® Omega-7 500 and 700 are not intended to produce any physical or other technical effects that are relevant to safety of the of the ingredient.

# Part 3: Dietary Exposure

## 3.1 Intended Use

AlaskOmega® Omega-7 500 and 700, manufactured in accordance with current GMP, are intended to be used as nutritive ingredients in food to replace other dietary fats and oils.

The ingredients are not intended for use in foods where standards of identity would preclude such use, infant formula, meat, poultry, egg products, catfish, or any products that would require additional regulatory review by USDA.

## 3.2 Exposure Estimates

Exposure to AlaskOmega® Omega-7 500 and 700 from the intended use described above was estimated based on complete dietary fat replacement for the U.S population (ages 2+) using food consumption data from the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Surveys (NHANES). Fat concentrations were assigned to all relevant NHANES (2013–2014) food codes using composition data from the United States Department of Agriculture (USDA) Food and Nutrient Database for Dietary Studies (FNDDS). The FNDDS database provides information on the concentration of approximately 60 food constituents (including total fat and specific fatty acids) for each NHANES food code and accounts for both naturally occurring and added fat levels in food. The fat exposure data was then derived using analysis by Creme Food Safety software 3.6 (www.cremeglobal.com).

The WWEIA/NHANES 2013–2014 survey contains data from two non-consecutive 24-hour dietary recall interviews of 7,574 individuals. Creme software is a probabilistic modeling tool that uses high-performance computing to predict intake (including total aggregate exposure) of food codes or groups and/or individual food ingredients. Creme Food Safety performs calculations using large-scale food consumption data sets. It bases the calculated estimates on each individual's body weight from the survey, as opposed to averaged body weights. Calculations also incorporated the NHANES assigned sample weights for each individual in the survey, which measure the number of people in the population represented by that specific subject and help to ensure that the results statistically represent the entire U.S. population. Sample weights for NHANES participants incorporate adjustments for unequal selection probabilities and certain types of non-response, as well as an adjustment to independent estimates of population sizes for specific age, sex, and race/ethnicity categories. The data is shown for the total population background fat exposure. Results are given as both absolute exposure (g/day), as well as exposure relative to body weight (g/kg bw/day). In addition, data is shown for relevant

subcategories of fatty acids for evaluation of effects of AlaskOmega® Omega-7 500 and 700 on background exposure to these relevant categories.

Data estimated directly from the NHANES short 2-day survey do not necessarily adequately represent individual usual long-term intake due to the large amount of random error. This is because it may not capture infrequent consumers. It assumes that subjects who consumed a product on a survey day consume it every day of the year, and it does not adjust for potential day-to-day variation in intake (i.e., intra-individual variation over time is not accounted for). Thus estimation of "usual" or "lifetime" exposure was also added to the model based on methodologies developed by Nusser et al., 1996, at Iowa State University.[3] These lifetime data are considered the most relevant data, as GRAS exposure estimates should be based on expected regular exposure over the lifespan. The technique of estimating usual/lifetime intakes relies on the ability to transform the input daily average data (from food consumers) into normality, which is tested using the Anderson-Darling test statistic within the Creme Global software.

Estimated background total fat, fat subcategory, and relevant fatty acid exposure results are shown in Tables 6 and 7 below for the total population (ages 2 years and older).

**Table 6.** Estimated Background Absolute Exposure to Fat and Relevant Fatty Acid Subcategories (g/day) by the U.S. Population (ages 2+)

| Fat Category | Total Population (age 2+ yrs) | Absolute Consumption (g/day) | | | | |
|---|---|---|---|---|---|---|
| | | Daily Average | | | | Lifetime 90$^{th}$% Exposure Estimates |
| | N (% of total) | Mean | Mean SE | 90$^{th}$% | 90$^{th}$% SE | |
| Total Fat | 7067 (100) | 77.58 | 0.7073 | 122.0 | 1.855 | 115.7 |
| Saturated Fat | 7067 (100) | 25.56 | 0.2527 | 42.87 | 0.5733 | 38.74 |
| Monounsaturated Fat | 7067 (100) | 26.78 | 0.2626 | 42.79 | 0.5212 | 40.41 |
| Polyunsaturated Fat | 7067 (100) | 17.85 | 0.1919 | 29.26 | 0.5298 | 27.49 |
| C16:0 | 7067 (100) | 13.81 | 0.1340 | 22.71 | 0.3725 | 20.85 |
| C16:1 | 7067 (100) | 0.9997 | 0.01090 | 1.741 | 0.02521 | 1.539* |
| C18:1 | 7067 (100) | 24.18 | 0.2454 | 39.18 | 0.5477 | 36.60 |
| C20:5 n-3 (EPA) | 6993 (99) | 0.02639 | 0.001147 | 0.05454 | 0.004832 | 0.05927 |
| C22:6 n-3 (DHA) | 6909 (98) | 0.05777 | 0.002238 | 0.1385 | 0.005787 | 0.1307 |

SE = standard error
Creme #254, 256, & 257
*Creme Warning -2048, "number of days per person should be constant for a Foods calculation", Lifetime data may still be used

**Table 7.** Estimated Background Exposure to Fat and Relevant Fatty Acid Subcategories Relative to Body Weight (g/kg bw/day) by the U.S. Population (ages 2+)

| Fat Category | Total Population (age 2+ yrs) | Consumption Relative to Body Weight (g/kg bw/day) | | | | |
|---|---|---|---|---|---|---|
| | | Daily Average | | | | Lifetime 90th% Exposure Estimates |
| | N (% of total) | Mean | Mean SE | 90th% | 90th% SE | |
| Total Fat | 7067 (100) | 1.230 | 0.01389 | 2.213 | 0.03946 | 2.144 |
| Saturated Fat | 7067 (100) | 0.4121 | 0.005185 | 0.7655 | 0.02118 | 0.7455 |
| Monounsaturated Fat | 7067 (100) | 0.4208 | 0.004806 | 0.7539 | 0.01579 | 0.7249 |
| Polyunsaturated Fat | 7067 (100) | 0.2806 | 0.003435 | 0.5144 | 0.008962 | 0.4922 |
| C16:0 | 7067 (100) | 0.2217 | 0.002781 | 0.4078 | 0.01018 | 0.3969 |
| C16:1 | 7067 (100) | 0.01550 | 0.0001824 | 0.02840 | 0.0004771 | 0.02632* |
| C18:1 | 7067 (100) | 0.3810 | 0.004497 | 0.6859 | 0.01081 | 0.6605 |
| C20:5 n-3 (EPA) | 6993 (99) | 0.0003825 | 0.00001580 | 0.0007740 | 0.00006305 | 0.0007968 |
| C22:6 n-3 (DHA) | 6909 (98) | 0.0008266 | 0.00003009 | 0.001979 | 0.00009388 | 0.001492 |

SE = standard error
Creme #254, 256, & 257
*Creme Warning -2048, "number of days per person should be constant for a Foods calculation", Lifetime data may still be used

Total 90th percentile lifetime absolute and relative to bodyweight exposure to AlaskOmega® Omega-7 500 or 700 based on total replacement of background dietary fat exposure are estimated to be 115.7 g/day (see Table 6) and 2.144 g/kg bw/day (see Table 7), respectively. This assessment is extremely conservative as it includes the assumption that 100% of fat consumed is from AlaskOmega® Omega-7 500 or 700.

Because of the large number and variety of fat containing foods, it is nearly impossible that an individual will randomly or intentionally consume a product containing AlaskOmega® Omega-7 500 or 700 every single time that he/she consumes a fat-containing food daily over a lifetime. While food labels will list the ingredient and may even highlight the ingredient occasionally in marketing, it is assumed that many consumers will not always realize that the ingredient is present in the food. In other words, it will be an "invisible" ingredient to many consumers, which decreases the chance that only food products that contain the ingredient will be chosen by those consumers. Additionally, there will be cost and market share limitations of adding this specialty ingredient to foods in general, making it even less likely that an individual would consume it in all foods consumed daily.

Furthermore, most foods contain fat as an endogenous constituent, and in many foods, fat is a major component (e.g., meat, fatty fish, dairy, eggs). Endogenous fats in such foods are excluded from substitution by AlaskOmega® Omega-7 500 or 700; in reality only foods containing added fat have the potential to contain this ingredient. Additionally, even if it were possible to replace endogenous fats in such foods, many of them are excluded from the intended use of AlaskOmega® Omega-7 500 or 700 by virtue of standards of identity and/or other categorical exclusions listed in Subparts 1.4 and 3.1. Thus, considering AlaskOmega® Omega-7 500 or 700 as total dietary fat replacement in our estimates is enormously conservative.

More realistic estimates of fat replacement can be calculated based on the assumption that AlaskOmega® Omega-7 500 or 700 will replace only 10% of dietary fat consumed. In light of the limitation to add AlaskOmega® Omega-7 500 and 700 only to foods that contain added fat and market share limitations due to the cost of the ingredient, market competition, and natural variation in food choices from day to day, this assumption is still considered highly conservative. This calculation results in lifetime 90$^{th}$ percentile AlaskOmega® Omega-7 500 or 700 consumption estimates of 11.57 g /day or 0.2144 g/kg bw/day, respectively, equivalent to approximately 8.329 g/day (0.1543 g/kg bw/day) palmitoleic acid if considering all exposure as from AlaskOmega® Omega-7 700 or to approximately 5.846 g/day (0.1083 g/kg bw/day) palmitoleic acid if considering all exposure as from AlaskOmega® Omega-7 500 (see Table 8 below). Relative to background palmitoleic acid exposure, 8.329 g/day represents a 531% increase.

Based on typical levels of fatty acids present in AlaskOmega® Omega-7 500 and 700 (see Table 1) the expected effects of the intended use of the ingredients on background exposure to fat subcategories and relevant individual fatty acids, at the 90$^{th}$ percentile lifetime exposure and assuming replacement of 10% of dietary fats, are shown in Table 8 below.

**Table 8.** Estimated Effects of Replacement of 10% of Dietary Fat with AlaskOmega® Omega-7 500 & 700 on 90th Percentile Fat and Relevant Fatty Acid Consumption

| Fat Category | Units | Lifetime 90th percentile Background Exposure Estimates* | Replacement of 10% of Dietary Fat at 90 Percentile Lifetime Consumption | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Exposure from AO®O-7 500 | AO® O-7 500 + Background | %Δ | Exposure from AO®O-7 700 | AO® O-7 700 + Background | %Δ |
| Total Fat | g/day | 115.7 | 11.57 | 115.7 | 0 | 11.57 | 115.7 | 0 |
| | g/kg bw/day | 2.144 | 0.2144 | 2.144 | 0 | 0.2144 | 2.144 | 0 |
| Saturated Fat | g/day | 38.74 | 2.381 | 37.25 | -3.85 | 1.210 | 36.08 | -6.88 |
| | g/kg bw/day | 0.7455 | 0.04413 | 0.7151 | -4.08 | 0.02243 | 0.6934 | -6.99 |
| Monounsaturated Fat | g/day | 40.41 | 6.523 | 42.89 | 6.14 | 8.333 | 44.70 | 10.62 |
| | g/kg bw/day | 0.7249 | 0.1209 | 0.7733 | 6.67 | 0.1544 | 0.8068 | 11.30 |
| Polyunsaturated Fat | g/day | 27.49 | 2.159 | 26.90 | -2.15 | 1.761 | 26.50 | -3.59 |
| | g/kg bw/day | 0.4922 | 0.04001 | 0.4830 | -1.87 | 0.03263 | 0.4756 | -3.37 |
| C16:0 | g/day | 20.85 | 2.355 | 21.12 | 1.29 | 1.208 | 19.98 | -4.21 |
| | g/kg bw/day | 0.3969 | 0.04363 | 0.4008 | 0.99 | 0.02239 | 0.3796 | -4.36 |
| C16:1 | g/day | 1.539 | 5.846 | 7.231 | 369.8 | 8.329 | 9.715 | 531.1 |
| | g/kg bw/day | 0.02632 | 0.1083 | 0.1320 | 401.5 | 0.1543 | 0.1780 | 576.3 |
| C18:1 | g/day | 36.60 | 0.6145 | 33.55 | -8.32 | 0 | 32.94 | -10.0 |
| | g/kg bw/day | 0.6605 | 0.01139 | 0.6058 | -8.28 | 0 | 0.5944 | -10.0 |
| C20:5 n-3 (EPA) | g/day | 0.05927 | 0.02314† | 0.07649 | 29.05 | 0.02314† | 0.05345 | -9.82 |
| | g/kg bw/day | 0.0007968 | 0.0004288† | 0.001146 | 43.82 | 0.0004288† | 0.0007185 | -9.82 |
| C22:6 n-3 (DHA) | g/day | 0.1307 | 0.02314† | 0.1408 | 7.71 | 0.02314† | 0.1177 | -9.82 |
| | g/kg bw/day | 0.001492 | 0.0004288† | 0.001778 | 18.74 | 0.0004288† | 0.001346 | -9.82 |

*Total Population (age 2+ yrs) from Tables 6 & 7

†Based on maximum specification limit of 0.2% (see Tables 2 & 3)

*Abbreviations:* %Δ, change in background exposure; AO®O-7, AlaskOmega® Omega-7; C16:0, palmitic acid; C16:1, predominantly palmitoleic acid; C18:1 predominantly oleic acid.

Calculations: AO®O-7 + background = 90% background + AO®O-7; %Δ = (((AO®O-7 + background)/background)-1) x 100%

From Table 8 above, the following additional observations and conclusions can be made with respect to the effects of the intended use of AlaskOmega® Omega-7 ingredients on consumption of fat subcategories and relevant fatty acids. Oleic acid is present at only low levels in AlaskOmega® Omega-7 (5.31% in the 500 ingredient and 0.00% in the 700 product based on the typical profile shown in Table 1). If 10% of fats and oils providing oleic acid intake were replaced by AlaskOmega® Omega-7 700, total oleic acid 90th percentile lifetime exposure would be reduced by 3.66 g/day resulting in a drop from 36.6 g/day to 32.9 g/day; however, the net effect on total monounsaturated fat exposure would be an increase of 4.29 g/day.

Considering a 20.35% palmitic acid content (based on the typical fatty acid profile in Table 1) of AlaskOmega® Omega-7 500, if 10% of fats and oils providing palmitic acid intake were replaced by AlaskOmega® Omega-7 500 total palmitic acid 90th percentile lifetime exposure would be increased by 0.269 g/day from 20.9 to 21.1 g/day; however, the net effect on total saturated fat exposure would be a decrease of 1.49 g/day.

If replacing 10% of fats and oils providing polyunsaturated fatty acids with AlaskOmega® Omega-7 700 (based on the typical profile from Table 1 as a worse case), the net effect would be a reduction in total daily polyunsaturated fatty acid intake of 0.988 g/day at the 90th percentile lifetime exposure from 27.5 to 26.5 g/day.

Although levels of EPA and DHA are very low in AlaskOmega® Omega-7 ingredients and limited by specification to a maximum of 0.2% each, background dietary exposure to EPA and DHA is also very low. As such, replacement of 10% of other dietary fats and oils with AlaskOmega® Omega-7 ingredients may result in fairly large percent changes; however, the estimated results, in terms of absolute effect, represent either slight increases or decreases in EPA and DHA intake. At the 90th percentile lifetime exposure, assuming EPA and DHA are both present in AlaskOmega® Omega-7 ingredients at the maximum level of 0.2% permitted by the ingredient specifications, exposure to EPA would increase by 17.2 mg/day from 59.3 to 76.5 mg/day, and exposure to DHA would increase by 10.1 mg/day from 130.7 to 140.8 mg/day if coming from AlaskOmega® Omega-7 500. If from AlaskOmega® Omega-7 700, EPA would decrease by 5.8 mg/day from 59.3 to 53.4 mg/day, and exposure to DHA would decrease by 12.8 mg/day from 130.7 to 117.9 mg/day. Assuming EPA and DHA are present at the typical levels shown in Table 1, replacement of 10% of dietary fats and oils with AlaskOmega® Omega-7 500 would result in an increase of 6.8 mg/day EPA and 10.1 mg/day DHA at the 90th percentile lifetime exposure while replacement of 10% of dietary fats and oils with AlaskOmega® Omega-7 700 would result in an increase of 2.2 mg/day EPA and a decrease of 10.8 mg/day DHA at the 90th percentile lifetime exposure. At the mean, the maximum estimate increase in EPA + DHA is 37.1 mg/day resulting in a combined increase in background exposure from 92.1 to 129.2 mg/day (data not shown). This level of exposure is well below the 3.0 grams/person/day of EPA and DHA combined that are considered protective of possible adverse effects by FDA. EPA and DHA exposure from AlaskOmega® Omega-7 ingredients are incidental rather than intentional exposures, and it is expected that companies specifically interested in adding EPA and/or DHA to foods, under the limitations set forth in regulation at 21 CFR 184.1472 would not choose AlaskOmega® Omega-7 ingredients as replacement oils in their products. It is further assumed that consumers interested in intentionally increasing their intake of EPA and DHA would specifically choose products containing oils high in EPA and DHA.

Overall, the net effect of replacement of 10% of other dietary fats and oils with AlaskOmega® Omega-7 ingredients could result in an increased consumption of 2.48–3.30 g/day of total unsaturated fatty acids while decreasing the consumption of saturated fatty acids by 1.49–2.66 g/day.

## Part 4: Self-limiting Levels of Use

There are no known inherent self-limiting levels of use.

## Part 5: Experience Based on Common Use in Food Prior to 1958

AlaskOmega® Omega-7 Palmitoleic 500 and 700 are derived from Alaska pollock, a member of the cod family (Gadidae), which has a long history of use in the human diet and has been among the world's most harvested fish, topping the most recent list in 2014.[2] Additionally, edible fats and oils from plants and animals have been consumed since ancient times. However, no qualitative and quantitative data concerning consumption of Alaska pollock oil or palmitoleic acid prior to 1958 was located. This GRAS conclusion for AlaskOmega® Omega-7 500 and 700 is based on scientific procedures, and thus, experience based on common use in food prior to 1958 is not considered as data and information necessary to form the basis for the conclusion.

# Part 6: Narrative

## 6.1 Absorption, distribution, metabolism, and excretion (ADME)

The normal body weight of mammals (including humans) is comprised of 5–25% lipids, of which up to 90% are present in triacylglycerols (TAG).[4] TAGs are comprised of three fatty acids (carboxylic acids with a hydrocarbon chain/tail) esterified to a glycerol backbone, and the majority of TAGs are present as stored energy reserves (approximately 400,000 kJ in a typical 70 kg human) in adipose tissue while smaller amounts act as shock absorbers around organs or circulate in the blood and lymph tissues to transport fatty acids and other lipids to and from tissues; additionally fat acts as an efficient thermal insulator. The primary sources of utilizable TAGs are dietary absorption of FFAs, mono- and diacylglycerols (MAG and DAG, respectively), and glycerol; de novo synthesis (primarily in the liver); and the adipose tissue storage depot.

Fatty acid ethyl esters, when ingested, are emulsified by peristalsis and bile salts during digestion. They are also hydrolyzed by pancreatic lipase. This hydrolysis yields free fatty acids and ethanol. The ethanol released in this process is considered insignificant. One study reported a theoretical yield of 1.3 grams of ethanol from the ingestions of 8 grams of ethyl oleic acid, a similar percentage as that found in common foods such as vanilla ice cream and orange juice.[5] The free fatty acids (FFA), once released from ethanol, are absorbed at the intestinal mucosa epithelium, and the hydrolyzed FFAs are resynthesized as TAGs within the enterocyte and secreted into the lymph system complexed to proteins as lipoprotein structures known as chylomicrons, which are comprised of approximately 86% TAG, for transport to peripheral tissues (e.g., heart, muscle, and adipose).[4] It has been demonstrated that although the hydrolysis of ester forms of fatty acids may occur at a slower rate than hydrolysis of TAG forms, the uptake of fatty acids into chylomicrons is similar when the fatty acids are given in either form.[6,7]

TAG synthesized in the liver by the de novo pathway are packaged in very low-density lipoproteins (VLDL), which contain about 51% TAG (and a different composition of apoproteins compared to chylomicrons), that are secreted in to the blood stream (also for delivery to peripheral tissues), and to a much lesser extent (about 6%) low-density lipoproteins.[4] TAGs in both chylomicrons and VLDL are hydrolyzed by the lipoprotein lipase enzyme at capillary surfaces within peripheral tissues for delivery of free fatty acids to tissue cells where, depending on tissue type, they are either resynthesized into TAGs for storage or oxidized for energy (glycerol is returned to the liver for recycling or catabolism).

Use of TAG stored in adipose tissue is under hormonal regulation. Hormones initiate a phosphorylation signaling cascade resulting in activation of hormone-sensitive lipase (a.k.a., TAG lipase) and subsequent hydrolysis of one of the outer

fatty acids from the glycerol backbone. This allows DAG lipase followed by MAG lipase to act sequentially resulting in the complete hydrolysis of the TAG molecule and liberation of three FFAs and one glycerol per TAG. FFAs move from the adipocyte to the blood stream by passive diffusion where they complex with albumin and are circulated to distant tissues for cellular uptake (also primarily by passive diffusion; released glycerol molecules are primarily taken up and catabolized in the liver).

Regardless of the proximate origin (e.g., ingestion, de novo synthesis, or liberation from storage), of FFAs, in addition to their utilization as an energy substrate or resynthesis in to TAGs, some contribute to the synthesis of phospholipids within local cell membranes where they serve structural, regulatory, or transport (e.g., lipoprotein components) roles.[4, 8]

Oxidation of FFA for energy takes place within the mitochondria via the β-oxidation pathway.[4] Metabolic activation of FFAs for β-oxidation requires the addition of coenzyme A (CoA) to the carboxylic acid head of the FFA, which is catalyzed by a fatty acyl-CoA ligase, resulting in the formation of an acyl-thioester CoA conjugate (or acyl-CoA). Short- and medium-chain fatty acids move freely through the mitochondrial membrane and interact with their specific ligases within the mitochondrial matrix. However, FFAs with 13 or more carbons in their hydrocarbon tail require a specific transport system due to the impermeability of the inner mitochondrial membrane to long-chain fatty acids (LCFA) and acyl-CoAs. LCFA specific ligases are present in the outer mitochondrial membrane, where an acyl-CoA is formed and then enters the carnitine shuttle system in which carnitine acyltransferase I and carnitine acyltransferase II enzymes, respectively, replace CoA with carnitine on the outer membrane (forming an intermediate fatty acyl-carnitine) and carnitine with CoA on the inner membrane releasing the acyl-CoA within the matrix. Carnitine acyltransferase I also plays a role in regulation of fatty acid metabolism as it is strongly inhibited by the first intermediate product in de novo fatty acid synthesis; thus, shutting down β-oxidation of LCFA under conditions that favor synthesis.

β-oxidation is a four-reaction cycle, as described below (see Figure 3) with each cycle shortening the acyl chain by two carbons and in so doing, releasing a molecule of acetyl-CoA (which then enters the citric acid cycle and is fully oxidized to carbon dioxide ($CO_2$)) and generating of one molecule each of reduced flavin adenine dinucleotide ($FADH_2$), and reduced nicotinamide adenine dinucleotide (NADH). The pathway name, β-oxidation, is derived from the fact that each step begins with the oxidation of the β-carbon. As an energy source, fatty acids yield 37 kJ/g (or approximately 8.2 adenosine triphosphates (ATP) for every full oxidation to $CO_2$), more than double that yielded from carbohydrate or protein, and are the major energy source for most cells (brain cells are a notable exception under typical American dietary conditions).

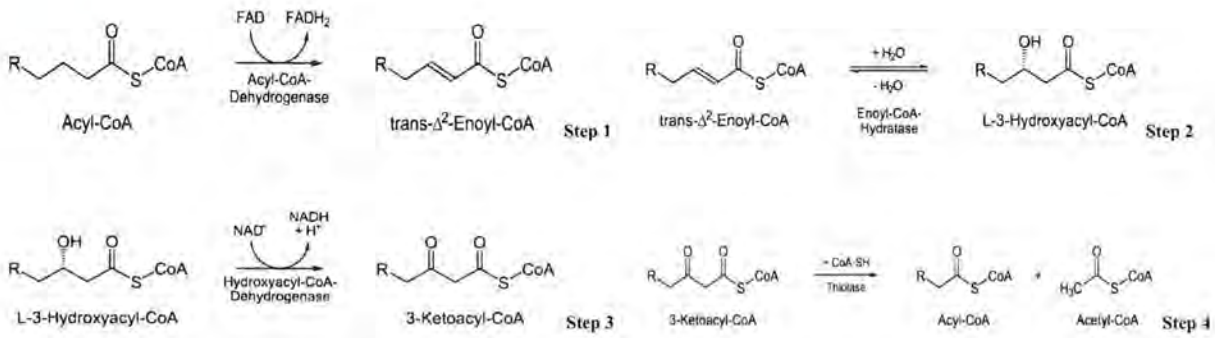


**Figure 3.** β-Oxidation Cycle

**Step 1:** Dehydrogenation. A fatty acyl-CoA is dehydrogenated between the α- and β-carbons by fatty acyl-CoA dehydrogenase, resulting in the formation of a *trans*-Δ2-enoyl-CoA and reduction of FAD to give $FADH_2$.

**Step 2:** Hydration. An enoyl-CoA hydratase catalyzed hydration of the *trans*-Δ2-enoyl-CoA β-carbon produces the Step 2 intermediate product, L-3-hydroxyacyl-CoA.

**Step 3:** Dehydrogenation. Next, 3-hydroxyacyl-CoA dehydrogenase catalyzes an $NAD^+$-dependent dehydrogenation of the newly formed 3-hydroxy group to give a 3-ketoacyl-CoA and NADH.

**Step 4:** Thiolytic cleavage. In the final reaction of the cycle, cleavage of the α—β bond by the thiol group of a second CoA molecule (CoA-SH) is catalyzed by β-ketothiolase, releasing acetyl-CoA.

The above cycle continues with the newly formed acyl-CoA, now two carbons shorter than its starting length, until the acyl-CoA is fully oxidized, assuming the starting FFA was fully saturated or contained only *trans* double bonds. In the case of *cis*-unsaturated fatty acids, such as DHA, β-oxidation proceeds until a *cis* double bond is reached at the Δ3 or Δ4 carbon of the newly formed acyl-CoA product, at which point additional reactions are required as the enoyl-CoA hydratase enzyme can only act on *trans* double bonds. These reactions are catalyzed by enoyl-CoA isomerase and 2,4-dienoyl-CoA reductase.

When the previous β-oxidation cycle results in the formation of a *cis*-Δ3-enoyl-CoA, enoyl-CoA isomerase converts it to a *trans*-Δ2-enoyl-CoA and β-oxidation then proceeds from Step 2. When the previous β-oxidation cycle results in the formation of a *cis*-Δ4-enoyl-CoA, Step 1 of the next β-oxidation cycle results in a *trans*-Δ2-*cis*-Δ4-dienoyl-CoA that is acted on by 2,4-dienoyl-CoA reductase to

produce *cis*-Δ3-enoyl-CoA, which then undergoes the enoyl-CoA isomerase reaction described above.

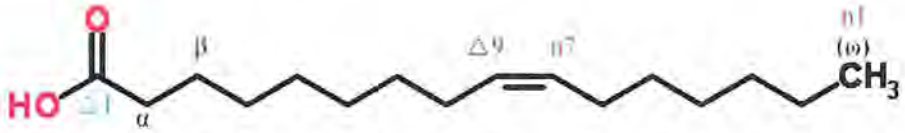


**Figure 4. Palmitoleic Acid Numbered Structure (linear depiction)**

Thus, in the case of palmitoleic acid (C16:1, *cis*-$\Delta^9$), with its first double bond at the Δ9 position (see Figure 4), three cycles of β-oxidation are carried out until the *cis* double bond is encountered at the newly formed 10-carbon enyl-CoA Δ3 position (i.e., C10, *cis*-Δ3-enoyl-CoA). At that point, the enoyl-CoA isomerase reaction transforms the *cis* bond to a *trans* bond at the Δ2 position resulting in formation of a C10, *trans*-Δ2-enoyl-CoA and the forth β-oxidation cycle proceeds through Steps 2, 3, and 4 to yield an eight-carbon acyl-CoA. β-oxidation of palmitoleic acid is completed with three more cycles resulting in the total generation of eight acetyl-CoAs to enter the citric acid cycle.

In the case of FFAs with odd-numbered carbon chains, the substrate of the final β-oxidation cycle is a five-carbon acyl-CoA, resulting in the release of one acetyl-CoA and one propionyl-CoA (3 carbons). Propionyl-CoA is carboxylated by propionyl-CoA carboxylase to form D-methylmalonyl-CoA that is converted by methylmalonyl-CoA epimerase to its stereoisomer L-methylmalonyl-CoA that is then converted to the citric acid cycle substrate, succinyl-CoA, catalyzed by L-methylmalonyl-CoA mutase.

Because of the current popularity of various low carbohydrate diets, a discussion of metabolization of fat for use as fuel would not be complete without a brief mention of the ketogenic pathway. This pathway is also prominent during starvation or intentional prolonged fasting states. When acetyl-CoA enters the citric acid cycle, it combines with oxaloacetate to form citrate and drive the cycle. When dietary carbohydrates are limited or unavailable the supply of citric acid cycle intermediates becomes limited resulting in reduced flux through the cycle and accumulation of acetyl-CoA derived from β-oxidation. When levels of acetyl-CoA rise, the β-ketothiolase reaction (Step 4 of β-oxidation) reverses resulting in generation of acetoacetyl-CoA from two acetyl-CoAs. The β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) synthase enzyme catalyzes the formation of HMG-CoA by the addition of a third acetyl-CoA to acetoacetyl-CoA, which, within the mitochondria, is split by HMG-CoA lyase to form acetoacetate and acetyl-CoA. Acetoacetate may be reduced to form D-β-hydroxybutyrate in an NADH-dependent reaction catalyzed

by β-hydroxybutyrate dehydrogenase or in small amounts, may spontaneously degrade to yield acetone. Collectively, acetoacetate, β-hydroxybutyrate, and acetone are referred to as ketone bodies. Ketogenesis occurs primarily in the liver and the ketone bodies are then transported to peripheral tissues where they can be utilized for energy production.

Palmitoleic acid is thought to be readily absorbed, and consuming foods rich in palmitoleic acid causes a significant increase in plasma palmitoleic acid. One study indicated that a 4% increase of energy consumed from dietary palmitoleic acid content equated to a 60% increase in plasma palmitoleic acid.[9] The metabolism of palmitoleic acid was investigated in rats and found to be similar to that of oleic acid in terms of incorporation into triglycerides, disappearance from blood, tissue distribution, oxidation, and recirculation.[10] In the study, radio-labeled palmitoleic and palmitic acid were administered together via intravenous injection, and with respect to palmitic acid (like oleic acid in previous work by the same author), palmitoleic acid was removed from the blood and oxidized more rapidly; however, the author noted an earlier study (by a different team of investigators) found no difference in extraction rates of saturated (i.e., palmitic) and unsaturated (i.e., oleic and linoleic) fatty acids and speculated the contrasting results might be due to methodological differences (fatty acids were injected into rats dissolved in whole rat serum while the cited work of the other investigators injected fatty acids bound to human albumin into rats). Hepatic elongation of palmitoleic acid to *cis* vaccinic acid was not extensive and did not affect interpretation of the data, and palmitoleic acid, similarly to oleic acid, was preferentially incorporated into neutral lipids (i.e., triglycerides) versus phospholipids.

### 6.1.1 Endogenous Metabolism and Distribution of Palmitoleic Acid

In additional to dietary exposure, palmitoleic acid is a product of endogenous lipogenesis within the human body. Palmitoleic acid is primarily synthesized in the liver. There it is used in the formation of triglycerides, packaged in very low-density lipoprotein (VLDL) and secreted into the blood.[11] De novo synthesis of monounsaturated fatty acids palmitoleic (C16:1, *cis*-$\Delta^9$) and oleic (C18:1, *cis*-$\Delta^9$) acids from saturated fatty acids palmitic (C16:0) and stearic (C18:0) acids, respectively, is catalyzed by stearoyl-coenzyme-A desaturase (SCD) enzymes. These unsaturated fatty acid SCD products become substrates for the formation of triglycerides.

In a radio-label study in humans, metabolism of algal derived palmitic and stearic acids was evaluated for seven days following administration of the labeled substance in an emulsion on an empty stomach.[12] Fasting subjects were administered either labeled palmitic or stearic acid, and a standardized diet was provided on Day 1 while subjects consumed their normal diets on Days 2–7. In

subjects administered palmitic acid, approximately 2% was desaturated to palmitoleic acid (peak concentration four hours post administration) while approximately 6% was elongated to stearic acid (peak concentration 8–12 hours post administration). In contrast, in subjects administered stearic acid, approximately 14% was desaturated to oleic acid while approximately 2% was shortened to palmitic acid with both peaking 8–12 hours post administration. Distribution and metabolism of palmitic and stearic acids, respectively, were variable across lipoprotein fractions although chylomicrons represented the most important fraction for each saturated fatty acid and its monounsaturated SCD product. Overall, desaturation of palmitic and stearic acids, while varying seven-fold, was minor and unlikely to have much impact on plasma fatty acid profiles relative to the wide variation in dietary fatty acid profiles in humans. The authors noted that higher percentages of desaturation observed in rodent studies are due in part to a higher SCD activity in rodents compared to humans and in part to the fasted/fed stated of rodents in various studies as diet significantly impacts hepatic SCD activity.

De novo synthesis of palmitoleic acid can also occur via beta-oxidation of other fatty acids. Consumption of a fatty acid emulsion containing 700 mg radiolabeled alpha-linolenic acid (C18:3, all-*cis*-$\Delta^{9, 12, 15}$) in a milkshake along with a standard breakfast resulted in enrichment above baseline of plasma phosphatidylcholine palmitoleic acid levels in men and women, which remained elevated for 21 days.[13] The highest concentrations were seen from 24–72 hours after ingestion. Plasma triacylglycerol palmitoleic acid was also enriched above baseline; however, this occurred only in men with peak concentrations occurring between 7 and 14 days. This study indicates that beta-oxidation (catabolism) of alpha-linolenic acid can result in the formation of palmitoleic acid in humans although palmitic acid was the major fatty acid synthesized.

Palmitoleic acid can be found in the plasma, lipids, red blood cell phospholipid bilayer, adipose tissue, brain, retina and viscera of most, if not all, mammals.[13-19] It is also found in human breast milk where it is estimated to make up 2% of the total fatty acid composition.[20] Adipose and liver tissue exhibit the highest concentration of palmitoleic acid found in the human body, and it appears to be a highly regulated fatty acid in adipose tissue, affecting systemic metabolism.[21] Some evidence points to a possible role of circulating palmitoleic acid as a fatty acid regulator and/or lipokine (lipid hormone) that is directly involved in the regulation of lipid and glucose metabolism.[16, 21] Palmitoleic acid is also considered the "parent" omega-7 fatty acid and, as such, is converted into other members of the omega-7 family, such as *cis*-vaccinic acid (C18:2, *cis*-$\Delta^{11}$) although this conversion has only been reported to occur in plants.[14]

## 6. 2 Toxicology Studies

Toxicological studies on AlaskOmega® Omega-7 500 and 700, the finished products, have not been conducted, and toxicological studies on pure palmitoleic acid were not found in the public domain. The absence of such studies is most likely due to the fact that this fatty acid, as well as the other fatty acids that constitute AlaskOmega® Omega-7 500 and 700, are considered "edible" and have an extensive history of consumption in foods.

There have, however, been a few studies conducted on similar ingredients or products, and these are described below. Of these, a battery of toxicological studies conducted by Collins et al. investigated an algal oil containing a high concentration of palmitoleic acid.[22] The test item was an ethyl ester oil derived from *Nannochloropsis* sp. (a marine microalgae) and standardized to contain 20–25% palmitoleic acid, 23–30% EPA, and no DHA, and is referred to below as Algal-EE oil. A comparison of overall fatty acid profiles of AlaskOmega® Omega-7 ingredients and Algal-EE oil is shown in Table 8, below.

**Table 8:** Typical Fatty Acid Profiles—AlaskOmega Omega-7 and Algal-EE

| Fatty Acid Ethyl Esters | | AlaskOmega® Omega-7 500 (% w/w) | AlaskOmega® Omega-7 700 (% w/w) | Algal-EE oil (% w/w) |
|---|---|---|---|---|
| Common Name | Lipid numbers + $\Delta^X$ | | | |
| Myristic | C14:0 | 0.08 | 0.02 | 3.4 |
| Myristelaidic | C14:1, *trans*-$\Delta^9$ | 0.02 | 0.00 | 0.0 |
| Myristoleic | C14:1, *cis*-$\Delta^9$ | 0.48 | 0.03 | 0.0 |
| Pentadecanoic | C15:0 | 0.00 | 0.00 | 0.3 |
| Palmitic | C16:0 | 20.35 | 10.44 | 18.2 |
| Palmitoleic | C16:1, *cis*-$\Delta^9$ | 50.52 | 71.98 | 22.3 |
| Margaric | C17:0 | 0.00 | 0.00 | 0.2 |
| Stearic | C18:0 | 0.11 | 0.00 | 0.6 |
| *trans*-octadecenoic | C18:1, *trans* (isomer unidentified) | 0.93 | 0.00 | 0.0 |
| Oleic | C18:1, *cis*-$\Delta^9$ | 5.31 | 0.00 | 2.3 |
| *cis*-vaccenic | C18:1, *cis*-$\Delta^{11}$ | 2.01 | 0.00 | 0.5 |
| Linoleic | C18:2, all-*cis*-$\Delta^{9, 12}$ | 0.56 | 0.00 | 1.0 |
| Gamma-linolenic | C18:3, all-*cis*-$\Delta^{6, 9, 12}$ | 0.06 | 0.00 | 0.0 |
| Alpha-linolenic | C18:3, all-*cis*-$\Delta^{9, 12, 15}$ | 0.31 | 0.00 | 0.1 |
| Stearidonic | C14:4, all-*cis*-$\Delta^{6, 9, 12, 15}$ | 0.77 | 0.00 | 0.0 |
| Arachidic | C20:0 | 0.00 | 0.00 | 0.1 |
| Gadoleic | C20:1, *cis*-$\Delta^9$ | 0.04 | 0.00 | 0.0 |
| Gondoic | C20:1, *cis*-$\Delta^{11}$ | 0.02 | 0.00 | 0.2 |
| Arachidonic | C20:4, all-*cis*-$\Delta^{5, 8, 11, 14}$ | 0.00 | 0.00 | 4.3 |
| EPA | C20:5, all-*cis*-$\Delta^{5, 8, 11, 14, 17}$ | 0.11 | 0.07 | 23.9 |
| Docosanoic | C22:0 | 0.04 | 0.00 | 0.1 |
| Erucic | C22:1, *cis*-$\Delta^{13}$ | 0.00 | 0.00 | 0.1 |

| Fatty Acid Ethyl Esters | | AlaskOmega® Omega-7 500 (% w/w) | AlaskOmega® Omega-7 700 (% w/w) | Algal-EE oil (% w/w) |
|---|---|---|---|---|
| Common Name | Lipid numbers + $\Delta^X$ | | | |
| Docosapentaenoic | C22:5, all-*cis*-$\Delta^{7, 10, 13, 16, 19}$ | 0.03 | 0.00 | 0.0 |
| DHA | C22:6, all-*cis*- $\Delta^{4, 7, 10, 13, 16, 19}$ | 0.2 | 0.02 | 0.0 |

### 6.2.1 Bacterial Reverse Mutation Assays

Collins et al., conducted a GLP and OECD 471 complaint bacterial reverse mutation test of Algal-EE oil.[22] The bacterial tester strains were exposed to seven concentrations from 5–5000 µg/plate in the initial plate incorporation test and to 5 concentrations ranging from 50–5000 µg/plate in the confirmatory pre-incubation test. The vehicle and negative control was DMSO and appropriate strain specific positive controls for use with and without S9 metabolic activation were used. The negative control results were comparable to historical negative control ranges and the positive controls induced biologically relevant increases in revertant colonies. The test item did not exhibit cytotoxicity, and no biologically relevant increases in revertant colony numbers with respect to the negative controls were observed in any tester strain at any concentration with or without metabolic activation.

The mutagenicity of *Helianthus annuus* L. (sunflower) seed oil was evaluated according to the methods of Mortelmans and Zeiger, 2000 and OECD test guideline 471.[23] Linoleic acid, a polyunsaturated omega 6 essential fatty acid, was the predominant fatty acid present in the test item comprising 50.68% (w/w). Palmitic acid (8.58%) was the predominant saturated fatty acid in the test item and oleic acid (27.27%) was the predominant monounsaturated fatty acid. While palmitoleic acid was the second most predominant monounsaturated fatty acid, it comprised only 0.9% (w/w) of the test item. The test was conducted using the pre-incubation method using five concentrations of the test item ranging from 10–200 µL/plate (no dose justification was provided) with or without S9 metabolic activation. The negative control was phosphate buffer and strain specific positive controls for use with and without metabolic activation were used although justification for use of 4-Nitroquinoline *N*-oxide with tester strains *Salmonella typhimurium* TA97a, TA98, and TA100 without metabolic activation was not reported. Statistically significant increases in revertant colony numbers compared to controls were observed for all positive controls. Concentration-dependent cytotoxicity of the test item was observed in tester strains TA97a, TA98, and TA102 with metabolic activation and in strain TA100 without metabolic activation. Compared to negative controls, no statistically significant increases in revertant colony numbers or mutagenicity ratios ≥2 were observed at any test item concentration with or without metabolic activation.

### 6.2.2 In vitro Mammalian Chromosomal Aberration Assay

The clastogenic potential of Algal-EE oil was investigated in a GLP study conducted according to OECD test guideline 473.[22] Human peripheral blood lymphocytes were exposed to concentrations of 233.28, 388.8, 648.0, and 1080 µg/mL Algal-EE oil for 3 h with and without S9 metabolic activation and concentrations of 50, 100, 200, 400, 600, 800, and 1080 µg/mL Algal-EE oil for 21 h without S9. The high concentration of 1080 µg/mL was chosen due to excessive fluctuations in osmolarity at higher concentrations; the negative/vehicle control was ethanol and positive controls for use with and without metabolic activation were cyclophosphamide and mitomycin C, respectively. Under all test conditions, the positive controls induced statistically and biologically significant increases in chromosomal aberrations compared to negative controls while the negative controls did not product biologically relevant increases compared to historical negative control ranges. No cytotoxicity was observed following 3 h of treatment with the test item with or without S9 at any concentration. Some dose related cytotoxicity was observed following 21 h treatment, reaching 43% (relative mitotic index = 57%) at the high-dose. No statistically significant increases in chromosomal aberrations, polyploidy, or endoreduplicated metaphases were observed at any test item concentration under any experimental conditions.

### 6.2.3 In vivo Mammalian Micronucleus Assay

A GLP micronucleus assay was conducted in male CD1 mice at doses of 500, 1000, and 2000 mg/kg Algal-EE oil according to OECD test guideline 474.[22] Doses and sex were based on a preliminary toxicity study in male and female CD1 mice, in which 2000 mg/kg (the limit dose according to OECD 474) was the maximum tolerated dose. Corn oil served as the vehicle, and all doses were administered twice, approximately 24 h apart, by gavage at a volume of 20 mL/kg. The negative control (corn oil) was administered twice, approximately 24 h apart, by gavage at a volume of 10 mL/kg, and the positive control, mitomycin C, was administered by gavage at a volume of 12 mL/kg on a single occasion. Bone marrow samples were obtained from all animals 24 h following the respective final administrations. No clinical signs of toxicity were observed in any animals during the treatment and observation periods. The positive control induced statistically significant increases in micronucleated polychromatic erythrocytes (MPCE). In the test item treated groups, no statistically significant increases were observed in MPCE frequency compared to negative controls. There were no statistically significant reductions observed in the proportion of polychromatic to total erythrocytes in any group.

### 6.2.4 Acute Oral Toxicity Study

Algal-EE oil was investigated in an acute oral toxicity test in rats conducted in compliance with GLP and the United Kingdom Animals Act of 1986 and according to OECD test guideline 423.[22] The test item was dissolved in corn oil, and three healthy, nulliparous, non-pregnant female CD Sprague-Dawley albino rats were administered a single dose of 2000 mg/kg Algal-EE oil by gavage at a volume of 10 mL/kg as a limit test. Following 24 h observation for mortality and morbidity, using the same protocol, a second group was administered a single dose of 2000 mg/kg. All animals were observed frequently the first 24 h following dosing and at least twice daily for the following 14 days prior to sacrifice. Body weights were obtained prior to dosing and on Days 8 and 15 and weekly body weight gain was calculated. Following sacrifice by asphyxiation with carbon dioxide all animals were subjected to gross pathological examination. No mortality or clinical signs of toxicity were observed during the study, individual and group body weight gains were satisfactory, and no macroscopic abnormalities were observed at necropsy. The acute median $LD_{50}$ of Algal-EE was considered >2000 mg/kg bw.

## 6.3 Additional Scientific Studies

### 6.3.1 Human Studies

Palmitoleic acid was evaluated in a randomized double-blinded placebo-controlled clinical trial.[24] Sixty dyslipidemic (LDL $\geq$ 100 mg/dL) adults of stable weight with evidence of systemic inflammation (CRP levels between 2 and 5 mg/L) were randomized to receive capsules containing a double-distilled anchovy oil containing 52.50% palmitoleic acid (from which all EPA and DHA had been removed) or placebo capsules of identical size and appearance containing MCT oil. Each active treatment capsule contained 220.5 mg palmitoleic acid, and each subject took one capsule (active or placebo depending on randomization) per day with meals for 30 consecutive days during which subjects were asked to maintain a stable diet. All subjects completed the study. In the treatment groups adverse events reported were gastrointestinal distress in two to three subjects and a headache in one subject. No adverse events were reported in the control group. No adverse effects on serum lipids or hs-CRP occurred.

Thirty-four adult men with mildly to moderately elevated cholesterol were randomized to receive one of three dietary fat interventions for 11 weeks each using a cross-over design.[9] One of the three interventions was designed to be a high palmitoleic acid diet. Subjects were instructed on the use of a fat counter and how to maintain a background dietary fat intake of $\leq$ 15% of energy. An additional 25% of fat energy was provided daily aliquoted between a milk beverage and a margarine into which the test oils were incorporated. The high palmitoleic test oil was

formulated from macadamia oil and in the margarine aliquot was partially hydrogenated. The dietary energy contribution from palmitoleic acid provided by the high palmitoleic test oil supplements was 4.0% while the major contribution came from oleic acid (13.3%) and analysis of food diaries indicated subject compliance with the test diets (e.g., means of 4.13 and 16.9% of dietary energy from palmitoleic acid and oleic acid, respectively, on pooled analysis). During the high-palmitoleic arm, two subjects reported gastrointestinal discomfort; however, the effect was transient and was considered related to macadamia oil being an unfamiliar dietary component. No adverse effects on plasma lipids were observed.

In a pilot study, 70 free-living subjects were randomized to receive either 45 or 90 g of macadamia nuts (approximately 5.84 or 11.68 g of palmitoleic acid per day, respectively) or a regular diet for one month.[25] The 90 g macadamia nut diet provided 50% of daily energy as fat. In a subsequent randomized crossover controlled-diet study, 34 adult men and women ate either a typical American high saturated fat diet (37% of energy from fat), an American Heart Association Step 1 diet (30% of energy from fat), or a macadamia nut-based diet (37% energy from fat) for 30 days. Thirty subjects completed the study. No important adverse effects were reported in either the pilot or main studies; however, in the pilot study, transient minor gastrointestinal tract discomfort, typical of that commonly observed with major changes in daily consumption of fats, was commonly reported. No adverse effects on serum lipid concentrations (total cholesterol, LDL, HDL, and triglycerides) were observed following any of the macadamia nut diets.

Yang et al. investigated effects of sea buckthorn (*Hippophaë rhamnoides*) seed and pulp oil in subjects with atopic dermatitis (AD).[26] Forty-nine subjects were randomized to receive 5 g of one of the sea buckthorn oils or placebo (paraffin oil) daily for four months. The sea buckthorn pulp oil contained 25% palmitoleic acid (equivalent to 1.25 g daily), 33% palmitic acid, and 26% oleic acid as the major fatty acids while the seed oil contained 24% linoleic acid, 25% α-linolenic acid, and 19% oleic acid. Twenty-nine of the 49 enrolled subjects were excluded from evaluation due to non-compliance or clinical examination (no further explanation was provided as to what factors on clinical examination were cause for exclusion (but adverse events were not specifically mentioned) or the distribution of excluded subjects among the study groups). None of the treatments had adverse effects on plasma lipids or IgE or AD symptoms. There was no mention of any adverse reactions to the treatments or placebo. In a follow-on study by the same investigation group and using the same study design, the same test items were administered to 22 subjects with AD for 4 months.[27] No adverse events or effects were reported.

Supercritical carbon dioxide ($CO_2$) extracts of sea buckthorn berry oil (i.e., seed + berry pulp; SBBO) were investigated in three clinical trials. The test items in all three trials reported here appear reasonably similar or identical to one another; all

test items appear to be produced by the same manufacturer, and it is unclear whether the slightly different fatty acid profiles reported were due to the format of reporting, normal variation, further refinements of the manufacturer's ingredient, or production of multiple similar ingredients. The three studies are summarized below.

Twelve healthy normolipidemic adult male subjects were randomized to receive 5 g daily of an SBBO containing 17.3% palmitoleic acid (865 mg daily), 23.4% palmitic acid, 20.5% oleic acid, 17.9% linoleic acid, 11.4% α-linolenic acid, and 5.5% vaccinic acid or placebo (supercritical $CO_2$ extracted fractionated coconut oil containing medium chain fatty acids) for 4 weeks each in a crossover design.[28] Plasma lipid profiles were not adversely affected. Slight but statistically significant elevations were observed in plasma glucose in both the SBBO and placebo groups; however, values remained within the normal range and, therefore, the effect was not considered clinically significant. Eleven of the 12 subjects completed the trial, and no adverse events were reported.

An SBBO reported to contain a slightly higher concentration of palmitoleic acid (24%) was administered to 30 healthy adult females for three months at a daily dose of 2 g (480 mg palmitoleic acid).[29] The other major fatty acid components of the oil were palmitic acid (24%), oleic acid (14%), linoleic acid (18%), α-linolenic acid (13%), and vaccinic acid (5.5%). No adverse effects were reported.

In another trial (reported across two published articles), 100 otherwise healthy adults with dry eye symptoms were randomized to receive 2 g daily SBBO or placebo oil (coconut and palm kernel MCT oil) for 3 months.[30, 31] The SBBO contained palmitoleic acid (17.3%; equivalent to 346 mg daily), palmitic acid (16.9%), oleic acid (15.8%), linoleic acid (12.3%), α-linolenic acid (7.5%), and vaccinic acid (5.4%). Eighty-six of 100 randomized subjects completed the trial. The reasons for study withdrawal and distribution among groups of the 14 subjects that did not complete the trail were not reported. The authors reported that blinding was effective and no treatment-related side-effects occurred based on a balanced distribution of subjects in the active and placebo groups guessing that they were in the SBBO group following one month of treatment.

## 6.4 Authoritative Safety Opinions

### 6.4.1 United States Department of Agriculture

The USDA Dietary Guidelines for Americans state that a healthy U.S.-Style eating pattern includes about 12–14% of daily caloric intake (or 27 g in 2000-calorie diet) from oils (i.e., "fats that contain a high percentage of monounsaturated and polyunsaturated fats").[32] Depending on age and gender, the recommended intake of

oils ranges from 15 to 51 grams daily, reflecting the estimated calorie needs (1000–3200) of the different groups.

## 6.5 Allergenicity

Organic's AlaskOmega® Omega-7 500 and 700 do not contain or have added any of the eight major allergens (milk, egg, fish, Crustacean shellfish, tree nuts, wheat, peanuts, and soybeans) identified, and required to be disclosed in labeling, by the Food Allergen Labeling and Consumer Protection Act (FALCPA). The highly refined oils derived from Alaska pollock and soy (natural vitamin E) do not demonstrate a hazard to allergic individuals and are excluded from the definition of "major food allergen" and excepted from the requirements of FALCPA.

No reports of allergic reactions to palmitoleic acid or Alaska pollock oil were found in our investigations. We did locate a single case report of an allergic reaction associated with ingestion of 4 g daily (2 capsules twice daily) for four days of a prescription fish oil, Lovaza®, containing a mixture of predominately EPA and DHA fatty acid ethyl esters derived from several fish sources occurring in an individual with a documented seafood allergy.[33] The labeling of Lovaza® contains the following warning/precaution: "**Fish Allergy** LOVAZA contains ethyl esters of omega-3 fatty acids (EPA and DHA) obtained from the oil of several fish sources. It is not known whether patients with allergies to fish and/or shellfish, are at increased risk of an allergic reaction to LOVAZA. LOVAZA should be used with caution in patients with known hypersensitivity to fish and/or shellfish." Overall, however, as with all highly refined oils derived from major allergens, as evidenced by their exclusion from the FALCPA definition of "major food allergen," the potential for allergic reactions to AlaskOmega® Omega-7 500 and 700 to occur is considered very low.

## 6.6 History of Consumption

Edible fats and oils from plants and animals have been consumed since ancient times, are currently widely consumed, and are considered GRAS. Salad and other cooking oil consumption reached 52.5 pounds per capita per year in 2010, according to the USDA Oil Crops Yearbook.[34] AlaskOmega® Omega-7 500 and 700 are derived from Alaska pollock, which has a long history of use in the human diet and has been among the world's most harvested fish, topping the most recent list in 2014.[2]

Typical total daily intake of fat/oil (and hence edible fatty acids) by humans is approximately 78 grams at the mean and 116 g at the 90$^{th}$ percentile, of which approximately 24 and 39 grams, respectively, are oleic acid (see Table 6). Oleic acid

is the most widely distributed fatty acid in nature. Levels of oleic acid in animal and vegetable oils are in the 30–50% range and are as high as 80% in olive oil. A 1997 study demonstrated the wealth of data on the use and safety of edible oils in separate meta-analyses of two data sets (36 crossover and 32-parallel designs) from 68 studies of over 2800 subjects that consumed an average of 10–12 grams per day of edible fatty acids for mean durations of 7–19 weeks.[35] The most common test substance was a high EPA fish oil and the most common placebo was olive oil; however, other oils consumed included corn, safflower, and linseed (i.e., flaxseed).

Palmitoleic acid is found in a variety of food products, such as oils derived from animal products and agricultural products, including marine and tropical oils, as well as nuts and seeds, and is also a component of human breast milk. Therefore, it has a history of consumption in the human diet, and is reported to be the second most common dietary *cis*-configured monounsaturated fatty acid,[36] albeit a minor component [37, 38] at a mean intake of approximately 1 g per day (reported as total C16:1 isomers) in the United States population 2 years of age and older compared to approximately 25 and 28 g per day for oleic acid and total monounsaturated fat, respectively.[39]

Fish and fish oils contain a broad spectrum of fatty acids, including omega-7 fatty acids. Anchovy, trout, salmon, swordfish, and catfish are examples of fish known to contain the highest levels of palmitoleic acid; one 3 oz serving of Alaska pollock contains about 9 mg (0.01% (or approximately 1% of total fat content)) of palmitoleic acid (reported as total *cis*-C16:1 fatty acids).[40] However, this quantity is small compared to a serving of macadamia nuts.

Macadamia nuts and their oil contain one of the highest percentages (approximately 13%) of palmitoleic acid, and from one ounce of macadamia nuts (10–12 nuts or 28.35 g) an individual would consume approximately 3.7 g of undifferentiated C16:1 fatty acids (palmitoleic acid presumably being the predominant C16:1).[40] Based on National Health and Nutrition Examination Surveys, approximately one third of Americans reported consuming nuts on any one day (averaging 21 g/d and up to 57 g/day).[41] The United States is the world's largest consumer of macadamia nuts, and 2001–2002 domestic consumption was expected to reach 44,069 tons.[42] According to the U.S. Department of Agriculture, macadamia nut consumption per capita in the United States has risen from 0.07 lbs in 1980–1981 to 0.15 lbs in 2004–2005 and then fell slightly to 0.13 lbs in 2005–2007 and to 0.10 lbs in 2007–2008.[43]

Palmitoleic acid is also found in plants of the *Brassica* family (e.g., turnip, rapeseed (from which canola oil was derived), mustard greens, arugula, radish) at levels ranging from 0.06% to 0.31% of total relative fatty acid composition.[14] Sea buckthorn, a common Chinese herbal medicine, with documented use dating back to 618 AD,[44] may contain up to 39% palmitoleic acid in the berry pulp oil.[45]

A number of edible oils contain palmitoleic acid (Table 9). Of the edible oils listed, macadamia nut oil is closest in its concentration of palmitoleic acid to AlaskOmega® Omega-7 500 although it has a higher oleic acid concentration.

**Table 9**. Approximate levels of Palmitoleic Acid in Edible Oils*

| | |
|---|---|
| AlaskOmega® Omega-7 Palmitoleic 500 and 700 | NLT 50 and 70%, respectively |
| Macadamia nut oil[46, 47] | 17% |
| Menhaden oil[40] | 10.5% |
| Herring oil[40] | 9.6% |
| Cod liver oil[40] | 8.3% |
| Sardine oil[40] | 7.5% |
| Salmon oil[40] | 4.8% |
| Olive oil[40] | 1.3% |

*Data tables from USDA Nutrient Database identified the fatty acid(s) as C16:1, undifferentiated; data tables from Maguire et al. and Shahidi and Miraliakbari identified the fatty acid(s) only as C16:1 although the authors of both articles referred to it as palmitoleic acid in the text.

In human breast milk, palmitoleic acid is the predominate *cis*-configured C16:1 isomer comprising 68.3% of C16:1 isomers *cis*-Δ7 to *cis*-Δ14 that are present in the milk [approximately 67.4% of total C16:1 isomers as calculated by the Panel] and accounting for 1.88% of total human breast milk fatty acids.[48]

## 6.7 Past Sales and Reported Adverse Events

According to Organic Technologies, more than 15,000 kg (equivalent to 15,000,000 servings at the suggested daily intake of 1 gram (500 mg palmitoleic acid)) of AlaskOmega® Omega-7 Palmitoleic ingredients have been sold as of September 2018. Organic Technologies states that no adverse event reports associated with the consumption of this ingredient to date have been received by the company.

No FDA letters regarding concern for safety to companies that market products containing palmitoleic acid or Alaska pollock were located. A search of MedWatch, FDA's adverse event reporting program, FDA's Recalls, Market Withdrawals, & Safety Alerts search engine, and FDA's Center for Food Safety and Applied Nutrition Adverse Event Reporting System did not uncover any mention of

palmitoleic acid or Alaska pollock products. All databases were accessed on September 11, 2018.

## 6.8 Relevant GRAS Notices

Importantly, GRAS notice 494, listed in Table 10 below, was for the intended use of "a mixture of fatty acid ethyl esters derived from anchovy or menhaden oil and standardized to approximately 50% palmitoleic acid (hereafter referred to as FAEE)" as a replacement source of fatty acids in food at an estimated lifetime daily exposure level of 5 and 10 g palmitoleic acid at the mean and 90$^{th}$ percentile, respectively. FDA's Office of Food Additive Safety, in its response to GRAS notice 494, concluded, "the agency has no questions at this time regarding … conclusion that FAEE is GRAS under the intended conditions of use," which indicates that the agency found no current reason to raise a safety concern over potential absolute exposure of up to 10 g daily of palmitoleic acid.

**Table 10.** GRAS Notices

| | GRN 105 | GRN 131 | GRN 146 | GRN 332 | GRN 371 | GRN 494 |
|---|---|---|---|---|---|---|
| **Subject** | Fish Oil Concentrate (4.6% palmitoleic acid) | Betapol™, a triglyceride mixture composed of fatty acids present in edible oils and fats, primarily oleic and palmitic | Salmon oil | Refined Pine Nut oil (palmitoleic acid 0.2%; oleic acid NLT 22%) | Krill oil (4.9% palmitoleic acid; 12.1% oleic acid) | Fish oil ethyl esters from anchovy or menhaden (50% palmitoleic acid) |
| **Uses** | Same categories as menhaden oil in 21 CFR 184.1472 | Use in infant formula for both term and preterm infants at levels of up to 80% total fat intake | Same categories as menhaden oil in 21 CFR 184.1472 | Baked goods and baking mixes, beverages and beverage bases, breakfast cereals, dairy product analogs, fats and oils, grain products and pasta, milk and milk products, nuts and nut products, processed fruits and fruit juices, processed vegetables and vegetable juices, snack foods, soft candy, soups and soup mixes | Breakfast cereals, cheese, beverages (nonalcoholic), fruit juices, frozen dairy desserts, milk products, and medical foods | As a replacement source of fatty acids in the food categories found in 21 CFR §170.3 (n) |
| **Intended Level of Use** | 7.7 g g/person/day, or 354.2 mg palmitoleic acid/person/day | 80% total fat intake | Levels not to exceed EPA+DHA 3.0 g/day | 17.8 g/person/day, or 35.6 mg palmitoleic acid/person/day | 8.3 g/person/day, or 406.7 mg palmitoleic acid/person/day | 20 g/person/day, or 10 g palmitoleic acid/person/day |
| **Outcome** | FDA has no questions | FDA has no questions | FDA has no questions | FDA has no questions | FDA has no questions | FDA has no questions |
| **Date of Closure** | October 15, 2002 | December 4, 2003 | December 4, 2002 | September 24, 2010 | July 22, 2011 | December 16, 2014 |

## 6.9 Basis for the GRAS Conclusion

Organic Technologies' AlaskOmega® Omega-7 500 and 700 have been the subject of a thorough safety assessment as described above. The totality of evidence supporting safety is comprised of data and information that establish the safety of AlaskOmega® Omega-7 500 and 700 under the conditions of their intended use and data and information that is corroborative of safety. The general availability and general acceptance, throughout the scientific community of qualified experts, of the data and information that establish the safety of AlaskOmega® Omega-7 500 and 700 under their intended conditions of use establish the general recognition of this data and information. Together, the establishment of safety based on scientific procedures and its general recognition form the basis for Organic Technologies' conclusion of GRAS status of AlaskOmega® Omega-7 500 and 700 for their intended use.

### 6.9.1 Data and Information that Establish Safety

The scientific data, information, and methods forming the basis of this conclusion are:

- The establishment of identity, demonstrating AlaskOmega® Omega-7 500 and 700 are highly refined, molecularly distilled, purified mixtures of edible fatty acid esters derived from crude Alaska pollock oil. Alaska pollock (*Gadus chalcogrammus*, Pallas, 1814; synonym *Theragra chalcogramma* (Pallas, 1814) concentrated to contain ingredient specific minimum concentrations of palmitoleic acid and containing insignificant levels of EPA and DHA;
- The method of manufacture and specifications, demonstrating the safe production and the high quality control standards of AlaskOmega® Omega-7 500 and 700;
- Pharmacokinetic data demonstrating the well-known and accepted ways in which the body acts on edible fatty acids in general and palmitoleic acid specifically;
- The toxicological studies on similar edible oils, and in particular the battery of studies on Algal-EE oil demonstrating the absence of genotoxic potential and acute oral toxicity of Algal-EE oil:
    - Algal-EE oil was not mutagenic in a bacterial reverse mutation test up to 5000 µg/plate (equivalent to 1115 µg/plate palmitoleic acid);

- Algal-EE oil was not clastogenic in an in vitro mammalian chromosomal aberrations test at up to 1080 µg/mL (equivalent to 241 µg/mL palmitoleic acid);
- Algal-EE oil was not genotoxic in an in vivo mammalian micronucleus test at 2000 mg/kg (equivalent to 446 mg/kg palmitoleic acid);
- Algal-EE oil did not exhibit acute oral toxicity in rats at 2000 mg/kg (equivalent to 446 mg/kg palmitoleic acid);

- The human studies in which no significant adverse events were observed following exposures to as much as 11.68 g/day palmitoleic acid for 30 days and as much as 10.4 g/day palmitoleic acid for 11 weeks.

Because the ingredient is intended as a nutritive replacement for edible fatty acids, its use in foods will necessarily be at relatively high levels. It is not possible to test such uses in laboratory animals at doses many fold greater than the level of exposure in humans; nonetheless, animal and human studies have not resulted in cause for concern, and because of the food-like nature of the ingredient, being composed of fish-derived lipids, all of which are typical components of fats and oil commonly found in the human diet, AlaskOmega® Omega-7 500 and 700 are expected to be acted upon by the body through similar physiological processes as other edible oils, which are well known as described in Subpart 6.1. Additionally, consistent with USDA Dietary Guidelines for Americans, the net effect of replacement of other dietary fats and oils with AlaskOmega® Omega-7 ingredients could result in an increased consumption of total unsaturated fatty acids while decreasing the consumption of saturated fatty acids.

As such, AlaskOmega® Omega-7 500 and 700 present no cause for concern when consumed as a replacement for added dietary fats and oils. The totality of evidence supporting the safety of the ingredients as described in this Subpart supports a conclusion that the intended use of AlaskOmega® Omega-7 500 and 700 is reasonably certain to be safe.

### 6.9.2 Data and Information that is Corroborative of Safety

The safety of AlaskOmega® Omega-7 500 and 700 is corroborated by the history of human consumption of edible fats and oils, including those high in palmitoleic acid, the more than 15,000 kg of AlaskOmega® Omega-7 ingredients that have been sold in dietary supplements with no adverse events having been reported, and the GRAS notices for similar oils that received acknowledgement letters without questions from US FDA.

### 6.9.3 Common Knowledge Element

The scientific data, information, and methods herein reported, that provide the basis of this GRAS conclusion by scientific procedures are published and available in the public domain. Part 7 of this GRAS notice contains the citations for the published studies. These publicly available data and information fulfill the requirement of the GRAS standard for general availability of the scientific data, information, and methods relied on to establish the safety of AlaskOmega® Omega-7 500 and 700 for their intended conditions of use. The peer-review of the published studies and lack of Letters to the Editor or other dissenting opinions as well as the acceptance of established lipid pharmacokinetics and biochemistry into well accepted textbooks used to educate generations of scientists provide ample evidence of general recognition among qualified experts that there is reasonable certainty that consumption of AlaskOmega® Omega-7 500 and 700 for their intended use is not harmful. The general availability and acceptance of these scientific data, information, and methods satisfy the criterion of the GRAS standard that general recognition of safety requires common knowledge throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food that there is reasonable certainty that the substance is not harmful under the conditions of its intended use.

## 6.10 Data and Information that is Inconsistent with the GRAS Conclusion

Plasma and adipose palmitoleic acid concentrations have been associated with increased risk of obesity in humans (as have oleic acid concentrations),[11, 49] and plasma palmitoleic acid has also been associated with triglyceridemia[37] while studies in animals have demonstrated adipose palmitoleic acid concentrations are protective against diet-induced obesity.[11] Nonetheless, the human studies were observational and do not prove causation. Palmitoleic acid is frequently used in studies as a surrogate for SCD activity rather than oleic acid in order to control for dietary influences on the individual fatty acid due to the relatively small dietary intake of palmitoleic acid compared to oleic acid,[37] and observational studies in humans suggest that, indeed, high levels of palmitoleic acid in overweight subjects are due to endogenous production via this mechanism.[49] Additionally, in a study on adipose palmitoleic acid, a positive association between adipose desaturation indexes of SCD1 (16:1/16:0 and 18:1/18:0) and obesity was also observed, and the association of obesity and adipose palmitoleic acid was attenuated in subjects with low carbohydrate intake while in subjects with high intake it was increased. Because carbohydrate intake increases hepatic SCD1 activity to a large degree and may also a causal factor of obesity, it is a plausible hypothesis that increased plasma and

adipose palmitoleic acid are merely surrogate markers of carbohydrate-induced increases in hepatic SCD1 activity.

In Subpart 6.3.1, we summarized a study by Nestel et al., 1994 and concluded, "No adverse effects on plasma lipids were observed" in subjects administered a high palmitoleic acid diet. However, in their article, the authors asserted that palmitoleic acid "behaves like a saturated and not a monounsaturated fatty acid in its effect on LDL cholesterol" implying at worst that ingestion of palmitoleic acid raises LDL cholesterol and increases cardiovascular disease risk and at best that palmitoleic acid has a negative effect on LDL cholesterol.[9] The authors also stated, "palmitoleic acid also lowered HDL cholesterol when compared with palmitic acid ... Our present study shows that a quite modest substitution of palmitic acid (from palm oil) for oleic acid (from Trisun) results in a significantly increased LDL cholesterol concentration ... [and] reinforce the validity of earlier conclusions that dietary palmitic acid is likely to raise the plasma cholesterol concentration in hypercholesterolemic men ... palmitoleic acid appears to have a similar effect." Thus, our conclusion in Subpart 6.3.1 requires some additional clarification.

While the authors were reasonably careful in the wording they used, the above quoted extracts are, nonetheless, not born out by the data presented in the article. As described in Subpart 6.3.1, the palmitoleic acid intervention was comprised of supplemental macadamia nut-derived high palmitoleic acid oil. The other two interventions were palm oil and a high oleic Trisun oil. All interventions provided an additional 25% fat energy to a low fat background diet. The subjects were mildly to moderately hypercholesterolemic middle-aged men. At baseline, mean total, LDL, and HDL cholesterol were approximately 225 ± 24.0, 163 ± 24.0, and 41.8 ± 9.67 mg/dL, respectively, and mean triglycerides were approximately 105 ± 37.2 mg/dL. Interesting, however, that the authors made no comparisons of post intervention levels to baseline values; instead, they compared the results of the three different treatments to one another. Thus, while it cannot be known whether any treatment results significantly differed from baseline or were statistically similar (except as can be inferred based on statistically significant differences between treatments), it is noteworthy that all post treatment results for total cholesterol and LDL cholesterol were lower than the baseline levels and all treatment results for HDL cholesterol were higher than the baseline levels. For triglycerides the palmitoleic acid group was higher than baseline (113 ± 44.0) while the other groups were lower than baseline values.

The high oleic acid intervention resulted in total and LDL cholesterol results that were statistically significantly lower than both the palmitoleic and palmitic acid interventions (thus, as both palmitoleic and palmitic were lower than baseline, it can be inferred that at least the oleic acid group was statistically significantly lower than baseline). For HDL cholesterol the palmitic acid treatment group was statistically significantly higher than the palmitoleic acid group (thus, it can be inferred that the

palmitic acid group was significant compared to baseline, but no inferences can be made for the oleic acid group). There were no statistically significant differences between the groups for triglycerides, and therefore, no inferences to baseline can be made.

Based on these results, no effects of palmitoleic acid on any lipid fractions can be stated or inferred. However, if there were any effects of palmitoleic acid on total, LDL, or HDL cholesterol they could only have been in the beneficial directions. In terms of the effect of oleic acid, the authors correctly inferred, "Our results, therefore, imply that palmitoleic acid, at least in the amount eaten, is less potent than oleic acid in depressing LDL receptor activity." It is additionally worth noting that part of the dietary interventions was given as a margarine in which the test oils had been partially hydrogenated. This could have imparted a negative bias in the observed results. For example, in 2015 FDA withdrew the GRAS status of partially hydrogenated oil (to take effect June 2018) stating, "the Food and Drug Administration (FDA or we) has made a final determination that there is no longer a consensus among qualified experts that partially hydrogenated oils (PHOs), which are the primary dietary source of industrially-produced *trans* fatty acids (IP–TFA) are generally recognized as safe (GRAS) for any use in human food ... the scientific evidence, including combined analyses of multiple studies (meta-analyses), supports a progressive and linear cause and effect relationship between *trans* fatty acid intake and adverse effects on blood lipids that predict coronary heart disease risk, including LDL–C, high-density lipoprotein cholesterol (HDL–C) and ratios such as total cholesterol (total-C)/ HDL–C and LDL–C/HDL–C.[50]" That dietary palmitoleic acid does not cause adverse effects on blood lipids or inflammatory markers of cardiovascular disease risk is further evidenced by the additional clinical trials using high-palmitoleic acid interventions cited and discussed in Subpart 6.3.1 as well as a study in F1B Golden Syrian Hamsters in which a 10% fat diet enriched with macadamia nut oil providing 20% of total fatty acids as palmitoleic acid did not have adverse effects on total, LDL, or HDL cholesterol; triglycerides; aortic total, free, and esterified cholesterol; liver weights; or liver lipid concentrations.[38]

## 6.11 Information that is Exempt from Disclosure under FOIA

There is no data or information in this GRAS notice that is considered exempt from disclosure under FOIA as trade secret or commercial or financial information that is privileged or confidential.

# Part 7: Supporting Data and Information

Initial literature searches were conducted during June and July 2016, and were renewed and supplemented during the course of time spanning October 2017 through February 2018. Additional literature searches were conducted on September 11, 2018.

## 7.1 Data and Information that are *not* Generally Available

The following data and information, relevant to the safety of the intended use of Organic Technologies' AlaskOmega® Omega-7 ingredients and discussed in Part 6 of this report, are not generally available:

- The sales data and absence of any adverse events reported to Organic Technologies since the market introduction of AlaskOmega® Omega-7 ingredients as dietary ingredients for use in dietary supplements as discussed in Subpart 6.7 of this report. While this information is corroborative of the safety of the intended use of AlaskOmega® Omega-7 ingredients, it does not form part of the basis for the safety conclusion as it provides no information as to the specific population(s) that consumed the ingredients or the amounts or durations of consumption on a per capita basis.

The above-identified information that is not generally available is corroborative information that is not absolutely necessary to establish the safety of the ingredients for their intended uses but was provided for the Panel's review pursuant to Subpart 1.9. We believe that qualified experts throughout the scientific community would be able to conclude that AlaskOmega® Omega-7 ingredients are not harmful under the conditions of their intended use without access to this corroborative information.

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**From:** Tim Murbach, ND, DABT
**To:** Kaneko, Kotaro
**Subject:** Re: GRN 000819
**Date:** Tuesday, April 02, 2019 5:38:38 PM
**Attachments:** image001.png

---

Thank you Dr. Kaneko,

We will get right to work on these. I will let you know if we have any questions or need clarification on anything after we have reviewed the attached.

Kind Regards,

Tim Murbach, ND, DABT
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On Tue, Apr 2, 2019 at 11:30 AM Kaneko, Kotaro <Kotaro.Kaneko@fda.hhs.gov> wrote:

> Dear Dr. Murbach,
>
> Please see attached a list of questions to be addressed for GRN 000819. Please send your responses within 10 business days in an email or separate document. Please do not send a revised notice.
>
> Please let me know if you have any questions.
>
> Best regards,
>
> Kotaro J. Kaneko, Ph.D.

Toxicologist/Chemist

Toxicology Review Team

Division of Biotechnology and GRAS Notice Review

Office of Food Additive Safety

Center for Food Safety and Applied Nutrition

Food and Drug Administration

Kotaro.Kaneko@fda.hhs.gov

(240) 402-5363

April 2, 2019

Dear Dr. Murbach,

After reviewing Wiley Organics, Inc./Organic Technologies' GRAS Notice GRN 000819 for the intended use of fatty acid ethyl esters from crude Alaska pollock oil, standardized to at least 500 mg/g (50%) or 700 mg/g (70%) palmitoleic acid, we noted the following questions that should be addressed. Responses may be sent in an email or in a separate document. Please do not send a revised copy of the notice.

We respectfully request a response within 10 business days. If you are unable to complete the response within that time frame, please contact me to discuss further options.

## General

1. Please provide the details of the published literature searches conducted in the preparation of this GRAS notice, including search criteria, database(s) used, and the dates searched.

   **RESPONSE:**

   The dates of literature searches are provided in Part 7 of the notice on page 49.

   The following databases were accessed during the literature searches:

   PubMed, ToxPlanet, TOXNET Databases (including HSDB, TOXLINE, ChemIDplus, DART), National Toxicology Program, Google Scholar, FDA's GRAS Notices Inventory Database, SCOGS, AIBMR Life Sciences' internal library, and Organic Technologies' internal library.

   The following search terms were used alone and/or in various Boolean combinations:

   Palmitoleic; palmitoleic acid; C16:1; (9*Z*)-Hexadec-9-enoic acid; *cis*-9-hexadecenoic acid; CAS RN 373-49-9; *Gadus chalcogrammus*; *Theragra chalcogramma*; Alaska pollock; walleye pollock; toxicity; toxicology; toxicity tests—subacute, subchronic, chronic, acute; mutagenicity; mutagenic; genotoxic; genotoxicity; genetic toxicity; clastogenic; carcinogenicity; carcinogenic; safety; no-observed-adverse-effect Level; NOAEL; no-observed-effect level; NOEL; chromosome aberrations; micronucleus; bacterial reverse mutations; Ames test; comet; pharmacokinetics; ADME; absorption; distribution; metabolism; excretion; elimination; bioavailability; biological availability.

   The exact Boolean search strings used at each of the accessed databases on each occasion of access were not documented. PubMed and Google Scholar were accessed multiple times using multiple search strings. ToxPlanet, TOXNET, and the GRAS notice inventory were also accessed multiple times using primarily key words related to the name of the substance rather than Boolean strings. SCOGS was accessed only once as it was not expected to change over time. AIBMR's internal library was searched only once as changes that occurred over time with respect to the substance were only the additions of documents located during the other searches.

## Chemistry

1. In Table 1 on page 9, you provided typical fatty acid profiles of AlaskaOmega Omega-7 500 and AlaskaOmega Omega-7 700.
    - Please briefly describe or cite (e.g., if AOAC or AOCS methods were used) the analytical method used.

      **RESPONSE:**

      The method used was Organic Technologies' internal method QC-193C, which employs analysis by gas chromatography with flame ionization detector (GC-FID). The internal method is based on AOCS Ce 1b-89 and is described in the attached document titled, *Organic Technologies Test Methods_QC-193C*.

    - Please briefly provide details of the samples analyzed (e.g., the number of batches sampled, the observed ranges, etc.).

      **RESPONSE:**

      The typical fatty acid profiles provided in Table 1 were generated from a single batch analysis each of AlaskaOmega Omega-7 (AO) 500 (Lot# [redacted] produced on 11-Oct-2017) and AO 700 (Lot# [redacted] produced on 8-Nov-2017).

      The values shown in Table 1 for palmitoleic acid, palmitic acid, EPA, and DHA are compliant with the corresponding specifications shown in Tables 2 and 3 and are reasonably consistent with the means of the corresponding fatty acids from the three batch analyses of AO 500 and two batch analyses of AO 700 reported in Tables 4 and 5, respectively, which are determined using the same analytical method. For purposes of comparison, these are shown in the table below (as Tables 2–5 reported the data as mg/g, they have been converted to percent wt/wt below for comparison with Table 1)

| Fatty acid | AO 500 Spec | Table 1 Data | Table 4 batch mean | AO 700 Spec | Table 1 Data | Table 5 batch mean |
|---|---|---|---|---|---|---|
| Palmitoleic | NLT 50.0% | 50.52% | 52.43% | NLT 70.0% | 71.98% | 71.00% |
| Palmitic | NLT 4.0% | 20.35% | 14.90% | NLT 5.0% | 10.44% | 9.25% |
| EPA | NMT 0.2% | 0.11% | 0.05% | NMT 0.2% | 0.07% | 0.05% |
| DHA | NMT 0.2% | 0.20% | 0.04% | NMT 0.2% | 0.02% | 0.02% |

2. In Section 2.2.1 (Manufacturing Overview), the notice does not describe how the standardization to 50% and 70% palmitoleic acid is achieved. Please describe, briefly, how the products are standardized to specific proportions of palmitoleic acid.

   **RESPONSE:**

   We start with a feedstock that contains a low concentration of palmitoleic acid and remove other fats through crystallization and distillation separation unit operations. We monitor the fatty acid composition during the manufacturing process and when we achieve target specifications (50% or 70% palmitoleic acid), the product is transferred to the packaging area. During the process, we may blend with other similar product streams that are concentrated in

palmitoleic acid to achieve the desired % of palmitoleic acid, to meet the specific product specification parameters.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,

Kotaro J. Kaneko, Ph.D.
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice review
Phone: 240-402-5363
Email: kotaro.kaneko@fda.hhs.gov

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| Quality Designee: (b) (6) 2/14/19 | | Quality Designee: (b) (6) 2/14/19 |

## 1.0 General Information

1.1 Purpose
This procedure is for the determination of the Omega 3 and Omega 7 fatty acid content of ethyl esters and triglycerides by GC/FID using n-butyl palmitate as an internal standard. Results are expressed as fatty acid equivalents and/or as ethyl esters in wt/wt percent.

1.2 Scope
This procedure applies to samples submitted to the QC Laboratory for fatty acid content of ethyl esters and triglycerides using n-butyl palmitate as the internal standard.

This method is based on AOCS Ce 1b-89 with the following variations:

1) n-butyl palmitate is used as the internal standard in place of C23:0.
2) Isohexane is used in place of isooctane.
3) Transesterification of triglycerides is performed using acetyl chloride.
4) Individual response ratios are established for Palmitoleic Acid Ethyl Ester, EPA Ethyl Ester and DHA Ethyl Ester.

1.3 Responsibility
Process Owner: Organic Technologies
Implementation: QC Chemists/Techs

1.4 Definitions
RPD: Stands for relative percent difference. The calculation is found in section 6.0 calculations
Lab Identification number: Sequential number used to track samples in the laboratory.
Primary: Solution that will be used to make other solutions.
Working: Solution that will be used in the analytical process.
Docosahexaenoic Ethyl Ester: Also known as DHA Ethyl Ester.
Eicosapentaenoic Ethyl Ester: Also known as EPA Ethyl Ester or Eicosapentaenote
Docosapentaenoic Ethyl Ester: Also known as DPA Ethyl Ester or Docosapentaenote
Palmitoleic Acid Ethyl Ester: Also known as Omega-7 Ethyl Ester
True mass: Actual mass of analyte present. Calculation is found in section 6.0 calculations.
OOS: Out of Specification. See Form8013-1 OOS Investigation and Form8013-3 OOS Decision Tree.

## 2.0 Specific Information

2.0 Interferences
No interferences have been found for this method.

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2.1 Safety

Acetyl chloride and Ethanol / Toluene are known to be a flammable and corrosive to skin and eyes on contact. Liquid or spray mist may produce tissue damage particularly on mucous membranes of eyes, mouth and respiratory tract. Skin contact may produce burns. Inhalation of the spray mist may produce severe irritation of respiratory tract, characterized by coughing, choking, or shortness of breath. Inflammation of the eye is characterized by redness, watering, and itching. Solvents should be kept away from heat/spark/open flames/hot surfaces and the container stored in a cool, dry, well ventilated location. Inhalation, skin and eye contact should be minimized. Sodium Carbonate is known to cause serious eye irritation. Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU) should be worn at all times.

2.3 Apparatus

Analytical Balance with tolerance of 0.0001 g
Balance, load capacity of 4Kg and tolerance of 0.1 grams
GC: HP 7890A equipped with HP 7693 Auto-injector/ sampler and HP Openlab Chemstation
Self-Zeroing 50.0mL volumetric burette
Disposable polyethylene Transfer Pipettes
2.0mL non-reactive GC vial w/ aluminum cap
100 mL Class A Graduated Cylinder (glass)
250 mL Class A Graduated Cylinder (glass)
1 L beaker (glass)
500mL beaker (glass)
25.0 mL Class A Volumetric Pipet (glass)
5.0 mL Class A Volumetric Pipet (glass)
1.0 mL Class A Volumetric Pipet (glass)

2.4 Reagents

2.4.1 Certified DHA Ethyl Esters Standard > 99.0 % pure DHA Ethyl Esters. This material is to be kept in the reagent refrigerator.
2.4.2 Certified EPA Ethyl Ester Standard > 99.0 % pure EPA Ethyl Ester. This material is to be kept in the reagent refrigerator.
2.4.3 Certified Palmitoleic Ethyl Ester Standard > 99.0 % pure Palmitoleic Ethyl Ester. This material is to be kept in the reagent refrigerator.
2.4.4 Distilled water
2.4.5 Acetyl chloride, ACS reagent grade
2.4.6 Ethanol/Toluene, 1:1. Made in the lab when needed. Using a graduated cylinder, add 250mL of anhydrous ethanol and 250mL of toluene (ACS reagent grade, water < 100 ppm) to a 1 L beaker. Transfer to a 1L glass jar. Add mole sieves and shake vigorously for 45 seconds. Stored in a flammable cabinet when not in use.
2.4.7 n-Butyl palmitate, 99%+ (internal standard)

2.4.8 5% sodium carbonate in water. Made in the lab when needed. Add 20 grams of sodium carbonate (ACS reagent grade) to a 500 mL beaker. Using a graduated cylinder, add 400 mL distilled water. Swirl until fully dissolved and transfer to a one-liter bottle for storage.

2.4 Waste Management
All solvent waste produced in this procedure is disposed of in the Flammable Waste container.

**3.0 Standards**

Calibration Standards: All calibration standards are prepared using Class A Volumetric Glassware. Calibration Standards are to be prepared fresh yearly. **Record the Weight and Purity of Palmitoleic Acid Ethyl Ester, DHA Ethyl Ester, EPA Ethyl Ester, and n-butyl palmitate; Dilutions Performed, solvent used, signed and dated recorded in a bound lab book.** All standards are stored in reagent refrigerator with Teflon caps when not in use.

Check Standards: All Check Standard Solutions are prepared using Class A volumetric glassware. Check standards are made from a different weighted sample than calibration standards. The Primary DHA Ethyl Ester/EPA Ethyl Ester/n-butyl palmitate check Standard is prepared fresh yearly. **Record the Weight and Purity of Palmitoleic Acid Ethyl Ester, DHA Ethyl Ester, EPA Ethyl Ester, and n-butyl palmitate; Dilutions Performed, solvent used, signed and dated recorded in a bound lab book.** All standards are stored in a 1oz. glass jars in the reagent refrigerator with Teflon caps when not in use.

3.1 Calibration Standards

3.1.1 Add 0.11 grams Palmitoleic Acid Ethyl Ester (weighed to 0.1mg), Add 0.11 grams EPA Ethyl Ester (weighed to 0.1mg) and 0.11 grams DHA Ethyl Ester (weighed to 0.1 mg) into a 1 oz. bottle.
3.1.2 Add 0.1 grams of n-butyl palmitate (weighed to 0.1mg).
3.1.3 Add 20 mL of isohexanes. Shake vigorously for 1 minute. Check for particulates and continue shaking for 1 minute intervals until completely dissolved.
3.1.4 Transfer 1.5 mL to a GC auto-injector vial.
3.1.5 Cap the vial and analyze per 5.0.
3.1.6 The standard is shot in triplicate and averaged.

3.2 Check Standard(s)

3.2.1 Add target weight in grams of each of Palmitoleic Acid Ethyl Ester, EPA Ethyl Ester, and DHA Ethyl Ester (weighed to 0.1 mg) into a 1 oz. bottle. Target weight for the Working Check Standard should be different than Calibration Standard weight (0.11 grams).
3.2.2 Add 0.1 grams of n-butyl palmitate (weighed to 0.1mg) (Same for each Check Standard).

3.2.3 Add 20 mL of isohexanes. Shake vigorously for 1 minute. Check for particulates and continue shaking for 1 minute intervals until completely dissolved.
3.2.4 Transfer 1.5 mL to a GC auto-injector vial.
3.2.5 Cap the vial and analyze per 5.0.
3.2.6 The check standard result is compared to the calculated expected result. Result should be within 2% Relative Percent Difference (RPD – see 6.0 Calculations). If not, perform and OOS investigation per procedure and repeat Calibration.

3.3 Linearity Check Standards
3.3.1 Prepare 3 Linearity Check Standards for each Analyte (Palmitoleic Ethyl Ester, EPA Ethyl Ester, and DHA Ethyl Ester). Determine the target weight for each Check Standard based on the 0.25 g sample size of the method to achieve roughly 10% (e.g 0.025 g), 45% (0.11 g) and 80% (0.20) expected assay. Add target weight in grams of each of Palmitoleic Acid Ethyl Ester, EPA Ethyl Ester, and DHA Ethyl Ester (weighed to 0.1 mg) into a 1 oz. bottle.
3.3.2 Add 0.1 grams of n-butyl palmitate (weighed to 0.1mg) (Same for each Linearity Check Standard).
3.3.3 Add 20 mL of isohexanes. Shake vigorously for 1 minute. Check for particulates and continue shaking for 1 minute intervals until completely dissolved.
3.3.4 Transfer 1.5 mL to a GC auto-injector vial.
3.3.5 Cap the vial and analyze in triplicate (3 injections of each Linearity Check Standard) per section 5.0.
3.3.6 Each set of 3 Linearity Check Standard results are entered into the *QC-193c Linearity Statistical Evaluation.xlsx* spreadsheet. The relative percent difference (RPD) of each triplicate injection should be less than 2%. The calculated $R^2$ for each set of 3 Linearity Check Standards for each analyte should be greater than 0.98. If not, perform and OOS investigation per procedure and repeat Calibration.

**4.0 Sample Preparation**

Record the sample number, date received, name of the sample prepared, the sample variety and all pertinent information on the label into the sample preparation log book. Record and report any abnormalities or damage.

4.1 When starting with Ethyl esters, proceed as follows
4.1.1 Add 0.25g of sample to a 1 oz. bottle.
4.1.2 Add 0.1 grams of n-butyl-palmitate (weighed to 0.1 mg)
4.1.3 Add 20 mL of iso-hexanes.
4.1.4 Vortex the sample for 40 seconds and analyze per section 5.0

4.2 When starting with triglycerides, proceed as follows

- 4.2.1 Add 0.25g of the sample into a 2 oz. bottle and record the weight to the nearest 1.0 mg.
- 4.2.2 Add 20 mL of 1:1 anhydrous ethanol/toluene.
- 4.2.3 Carefully add 0.5 mL of acetyl chloride. For samples with flavoring add 1.0 mL of acetyl chloride.
- 4.2.4 Cap the bottle, purge with nitrogen, vortex for 30 seconds and incubate at 80°C for 2 hours in order to complete the transesterification to the ethyl esters. Stir the samples every twenty minutes to facilitate transesterification.
- 4.2.5 Remove the bottle from the incubator and allow it to cool to room temperature (72-770F).
- 4.2.6 Add 10 ml of water containing 5% sodium carbonate, place cap with Teflon liner on bottle and vortex until neutralized. For samples containing flavoring use 20mL of water containing 5% sodium carbonate. Carefully remove the cap as neutralization may not be complete and the bottle will be under pressure. If neutralization is not complete, recap and vortex. Repeat until no pressure remains when opening the bottle.
- 4.2.7 Add 0.1 grams of n-butyl-palmitate (weighed to 0.1 mg) internal standard. Note: Using an analytical balance, weigh the n-butyl-palmitate by difference.
- 4.2.6 Mix for two minutes on a wrist action shaker or vortex vigorously for a minute.
- 4.2.9 Allow the layers to completely separate.
- 4.2.10 Add 0.5mL of the clear supernatant to a GC vial containing 1.0 mL of anhydrous ethanol.
- 4.2.11 Cap the vial, mix and analyze per section 5.0.
- 4.2.12 Repeat steps 4.2.1 – 4.2.12 to prepare a duplicate sample.

**5.0 Instrument**

GC-FID system. HP 7890A equipped with a Flame Ionization Detector (NPD), an HP 7693 series Auto-injector/sampler, and a split-splitless injector connected to a PC with HP-OpenLab Chemstation revision C.01.08 or higher.

Analytical Procedure:

**GC Method Name: QC-193c**

Method Information
Column – ReStek FameWax (30m x 0.25mm x 0.25µm film thickness); ReStek part 12497, or equivalent

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Injection Source: GC Injector
Injection Location: Front

OVEN

| | |
|---|---|
| Initial temp: | 180 'C (On) |
| Max temp: | 240 C |
| Initial time: | 01 min |
| Ramp1: | Rate 5 C/ min |
| | Final temp 240 C |
| | Hold time 07 min |
| Post temp | 180 C |
| Post time | 0 min |
| Run time | 20 min |

Inlet

| | |
|---|---|
| Mode | Split |
| Initial temp | 250 C |
| Pressure | 15.0psi |
| Split ratio | 60:1 |
| Split flow | 20.0 Ml/ min |
| Total Flow | 24.66 mL/ min |
| Gas savor | ON |
| Gas type | Helium |

Column

| | |
|---|---|
| Model # | Restek 12497 FameWax |
| Max temp | 250 C |
| Nominal length | 30 m |
| Nominal Film Thic | 0.25□m |
| Mode | constant flow |
| Pressure | 15.00 psi |
| Nominal initial flow | 1.664 mL/ min |
| Average velocity | 55.0 cm / sec |
| Outlet pressure | ambient |

Detector (FID)

| | |
|---|---|
| Temperature | 275 C |
| Hydrogen flow | 40.0 mL/ min |
| Air flow | 400 mL/ min |
| Mode | constant fuel and makeup |
| Makeup flow | 20.0 mL/ min |

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| | |
|---|---|
| Makeup gas type | Helium |
| Adjust offset | 30 |
| Electrometer | on |
| Bead | on |
| Equilibration time | 5.0 |

Injector

| | |
|---|---|
| Sample Washes | 4 |
| Sample wash vol | 8.0 µL |
| Sample pumps | 4 |
| Injection volume | 0.5 µL |
| Syringe Size | 10.0 µL |
| Postinj solvent A wash | 4 |
| Preing solvent A wash | 3 |
| Solvent A wash vol | 8µL |
| Postinj solvent B wash | 3 |
| Preinj solvent B wash | 3 |
| Solvent B wash vol | 8µL |
| Viscosity delay | 0.0 s |
| Plunger speed | 6000.0 |
| Pre injection dwell | 0.0 min |
| Post injection dwell | 0.0 min |

Signal

| | |
|---|---|
| Data rate | 20 Hz |
| Save Data | on |
| Zero | 0.0 off |
| Range | 0 |
| Fast peaks | off |
| Attenuation | 0 |

Integration Event Table

| | |
|---|---|
| Slope | 1 |
| Peak Width | 0.04 |
| Area Reject | 1 |
| Height rejects | 1.7 |
| Shoulders | off |

Calibration table

| | |
|---|---|
| Calculate | Internal Standard |
| Based on | Peak area |

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| | |
|---|---|
| Rel Reference Win | 0% |
| Abs ref Window | 0.2 min |
| Rel non-ref window | 0% |
| Abs non-ref window | 0.2 min |
| Multiplier | 1 |
| Dilution | 1 |
| Sample amount | 0 |
| Uncalibrated Peaks | not reported |
| Partial calibration | Yes, identified peaks are recalibrated |
| Correct all ret. Time | No, only for identified peaks |
| Curve type | Linear |
| Origin | included |
| Weight | equal |

Signal 1

| Fatty Acids (as Ethyl Esters) | Retention Time (min) |
|---|---|
| C10:0 Decanoic | 1.39 |
| C12:0 Dodecanoic | 1.78 |
| C14:0 Myristic | 2.51 |
| C14:1 Myristoleic | 2.71 |
| C16:0 Palmitic | 3.73 |
| C16:1 Palmitoleic | 3.89 |
| n-butyl palmitate (IS) | 5.27 |
| C18:0 Stearic | 5.50 |
| C18:1 Oleic | 5.64/5.75 |
| C18:2 Linoleic | 6.13 |
| C18:3 Linolenic (ALA) (n-3) | 6.78 |
| C18:3 gamma-Linolenic | 6.42 |
| C18:3 Calendic | |
| C18:4 Steridonic (STD) (n-3) | 7.11 |
| C20:0 Eicosanoic | 7.73 |
| C20:1 Gadoleic | 7.84/7.92 |
| C20:2 Eicosadienoic | 8.47 |
| C20:3 Eicosatrienoic (ETE) (n-3) | 9.25 |
| C20:4 Eicosatetraenoic (ETA) (n-3) | 9.52 |
| C20:4 Arachidonic | |
| C20:5 Eicosapentaenoic (EPA Ethyl Ester) | 9.76 |
| C22.0 Docosanoic | 10.21 |
| C22:1 Erucic | 10.34/10.42 |
| C22:2 Docosadienoic | 11.09 |

| | |
|---|---|
| C22:4 Adrenic | 11.55 |
| C22:5 Docosapentaenoic (DPA Ethyl Ester) | 12.37 |
| C22:6 Docosahexaenoic (DHA Ethyl Ester) | 12.64 |
| C24:0 Tetracosanoic | 12.77 |
| C24:1 Nervonic | 13.00 |
| C24:5 Tetracosapentaenoic | |
| C24:6 Tetracosahexaenoic | |

## 6.0 Calculations

6.0.1 Calculate the True Mass of the n-butyl palmitate internal standard and the Palmitoleic Ethyl Ester, EPA Ethyl Ester, and DHA Ethyl Ester standards in the calibration standard solution using the stated assay of the standards and the weights recorded during the preparation in step 3.1.

$$M_{x,r} = W_{x,r} \times P_{x,r}$$

where:

Wx,r = the weight of relevant standard (n-butyl palmitate, Palmitoleic Ethyl Ester, EPA Ethyl Ester, or DHA Ethyl Ester) added to the calibration standard solution
Px,r = the stated assay of the relevant standard (n-butyl palmitate, Palmitoleic Ethyl Ester, EPA Ethyl Ester, or DHA Ethyl Ester) used in the preparation of the calibration standard solution

6.0.2 Calculate the response ratio for Palmitoleic Ethyl Ester, EPA Ethyl Ester and DHA Ethyl Ester relative to the n-butyl palmitate internal standard. A minimum of three injections of the calibration standard is required. Once the area results are established, the True Mass calculated for each standard are entered into Openlab Chemstation to establish a revised method based on the calibration.

To calculate the response ratio manually, the average response ratio should be determined by averaging the individual response ratios $RR_r$ from each injection. The individual response ratios should not vary by more than 2%, relative (see calculation in 6.0.4, below).

$$RR_{x,r} = A_3 \times M_r / (M_3 \times A_{x,r}) \qquad RR_r = (RR_{1,r} + RR_{2,r} + RR_{3,r}) / 3$$

where:

$A_3$= area of the n-butyl palmitate internal standard in chromatogram of calibration standard solution

$A_{x,r}$= area of Palmitoleic Ethyl Ester, DHA Ethyl Ester or EPA Ethyl Ester (r) in chromatogram of calibration standard solution for each injection (x).
$M_3$= True Mass of n-butyl palmitate internal standard in calibration standard solution
$M_r$= True Mass of Palmitoleic Ethyl Ester, EPA Ethyl Ester or DHA Ethyl Ester (r) in calibration standard solution

6.0.3 Calculate the contained amounts of Palmitoleic Ethyl Ester, EPA Ethyl Ester and DHA Ethyl Ester present as fatty acid equivalent and as ethyl esters:

% Analyte (fatty acid equivalent) = $100 \times C \times RR_r \times A_x \times M_1 / (A_1 \times M_2)$
% Analyte (ethyl ester) = $100 \times RR_r \times A_x \times M_1 / (A_1 \times M_2)$

Calculate the contained amount of all other analytes present as fatty acid equivalent and as ethyl esters using a response ratio equal to the response ratio calculated above for EPA Ethyl Ester:

% Analyte (fatty acid equivalent) = $100 \times C \times RR_{EPA} \times A_x \times M_1 / (A_1 \times M_2)$
% Analyte (ethyl ester) = $100 \times RR_{EPA} \times A_x \times M_1 / (A_1 \times M_2)$

where:

$RR_{EPA}$ = Response Ratio of analyte
$A_x$ = Area of analyte
$A_1$ = Area of internal standard in test solution
$M_1$ = True Mass of Internal Standard (n-Butyl Palmitate) in test solution
$M_2$ = Weight of sample in test solution
C = Conversion from ethyl ester to fatty acid, per the table below:

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| Type | | Name | C Ratio |
|---|---|---|---|
| omega-7 | C16:1 | Palmitoleic | 0.901 |
| omega-3 | C16:3 | Hexadecatrienoic | 0.899 |
| omega-9 | C18:1 | Oleic | 0.910 |
| omega-6 | C18:2 | Linoleic | 0.909 |
| omega-3 | C18:3 | Linolenic (ALA) | 0.908 |
| omega-6 | C18:3 | gamma-Linolenic | 0.908 |
| omega-6 | C18:3 | Calendic | 0.908 |
| omega-3 | C18:4 | Steridonic (STD) | 0.908 |
| omega-9 | C20:1 | Gadoleic | 0.917 |
| omega-6 | C20:2 | Eicosadienoic | 0.917 |
| omega-6 | C20:3 | Dihomo-gamma-linolenic | 0.916 |
| omega-3 | C20:3 | Eicosatrienoic (ETE) | 0.916 |
| omega-3 | C20:4 | Eicosatetraenoic (ETA) | 0.916 |
| omega-6 | C20:4 | Arachidonic | 0.916 |
| omega-3 | C20:5 | Eicosapentaenoic (EPA Ethyl Ester) | 0.915 |
| | C22:0 | Docosanoic | 0.924 |
| omega-9 | C22:1 | Erucic | 0.923 |
| omega-6 | C22:2 | Docosadienoic | 0.923 |
| | C22:3 | Docosatrienoic | 0.923 |
| | C22:4 | Docosatetraenoic | 0.922 |
| omega-6 | C22:4 | Adrenic | 0.922 |
| omega-3 | C22:5 | Docosapentaenoic (DPA Ethyl Ester) | 0.922 |
| omega-6 | C22:5 | Docosapentaenoic | 0.922 |
| omega-3 | C22:6 | Docosahexaenoic (DHA Ethyl Ester) | 0.921 |
| | C24:0 | Tetracosanoic | 0.929 |
| omega-9 | C24:1 | Nervonic | 0.929 |
| | C24:2 | Tetracosadienoic | 0.929 |
| | C24:3 | Tetracosatrienoic | 0.928 |
| | C24:4 | Tetracosatetraenoic | 0.928 |
| omega-3 | C24:5 | Tetracosapentaenoic | 0.927 |
| omega-3 | C24:6 | Tetracosahexaenoic | 0.927 |

6.0.4 Relative Percent Difference – 2 readings

RPD = Absolute Value of $[(R_1 - R_2)/((R_1 + R_2) \times 2)]$

where:
$R_1$ = Reading 1, $R_2$ = Reading 2

Relative Percent Difference – 3 readings

RPD = Avg / Std Dev x 100

where:
Avg = $(R_1 + R_2 + R_3) / 3$
Std Dev = Square Root of $\{ [ (R_1 - Avg)^2 + (R_2 - Avg)^2 + (R_3 - Avg)^2 ] / 3 \}$
$R_1$ = Reading 1, $R_2$ = Reading 2, $R_3$ = Reading 3

**7.0 Quality Assurance Procedures**

7.1 Solvent Blank

Solvent Blank Injections of the **Extraction Solvents** are injected before any standard sequence is started. Solvent Blanks are also injected after any check standard is analysis. The blank must demonstrate 0.05ppm or less compounds present in the target reference area. If this amount is exceeded than the solvent blank injection must be performed again until it reaches an amount less than 0.05ppm. This step is making sure sample is not staying in the column and eluting on the next run giving you higher than expected results or mystery peaks.

7.2 Calibrations

Calibration curves are created using 45% concentration (see section 3.2) ran in triplicate and averaged. Linearity standard (see section 3.3) should be run after calibration is performed.

7.3 Check Standards

The Working Check Standard is implemented to ensure the integrity of the standards, validity of calibration curve and monitoring of the instrument's base line drift. The Working Check Standard is run after each calibration set.

7.4 Calibration Curve Validation Procedure

A calibration curve is Accepted if the Working Check Standard has a Relative Percentage Difference (RPD) less than 2% of the theoretical concentration on average of the post calibration linearity set.

If the Working Check Standard has a Relative Percentage Difference (RPD) greater than 2% then another check standard is prepared and analyzed. If the second check standard also has a Relative Percentage Difference (RPD) greater than 2% then the calibration set in question is Rejected and a fresh set of calibration standards is to be prepared and re-analyzed on the GC.

7.5 Corrective Actions

7.5.1 Unacceptable QC

Check standard not within 2% limit

1) Instrument will be checked for hardware problems then recalibrated with a fresh set of standard vials
2) If recalibration fails a fresh check standard will be made and new calibration will be performed with a fresh set of standard vials
3) If the fresh check standard fails a new set of calibration standards will be prepared, and a new calibration performed.
4) If the fresh check and calibration standards do not correspond an in-depth review of standard material, analysis method, and hardware will be performed.

7.5.2 RPD Greater than 2%

1) Samples will be reprocessed and analyzed.

**8.0 Reporting**

Minimum Reporting Levels of this method are {5% Palmitoleic Ethyl Ester, 5% EPA Ethyl Ester and 5% DHA Ethyl Ester}. The Maximum, reporting limits for this method are {90% Palmitoleic Ethyl Ester, 90% EPA Ethyl Ester and 90% DHA Ethyl Ester}, using the standard calibration set.

8.0.1 Computer Records

Results log: A file with quality assurance data, as well as all information recorded will be saved in computer format for an indefinite period.

**9.0 Instrument Maintenance**

Instrument maintenance is to be performed as dictated by chromatographic results. This maintenance is considered preventative to prolong the lifetime of the instrument and the integrity of analysis. All maintenance is to be recorded in the Instrument Maintenance Log.

9.0.1 Routine Maintenance includes

1) Replace Wash Solvent Vials A & B, refill wash solvents, check

gas pressures (He, Air & H2) and insure that the Detector is lit properly.
2) Replace the Inlet Septa, as needed.
3) Replace Inlet Liners and O-Ring (Inlet), as needed.
4) Cutting the ends of the Column, Replace Ferrules (column), Update the Retention Time in Openlab Chemstation, and Sonicate Detector Jets, as needed.
5) The installation of a new Column, as needed.

## 10.0 Appendix, References & Further Reading


AOCS official method Ce-1b-89, Fatty acid composition by GLC: Marine oils, Champaign IL, 1992.


## 11.0 Revision History

| | |
|---|---|
| 09/05/2012 | Change reporting in mg/g to reflect rounding |
| 10/03/2012 | Change sec 3.3 wording to "Emulsion Concentrate" |
| 11/08/2013 | Change sec 4.0, added system suitability check. |
| 03/21/2017 | Remove section dealing with emulsion samples; change to 0.25mm ID column. |
| 12/03/2018 | Document review and revision. Changed Eicosapentaenoic and Docosahexaenoic to reflect the Ethyl Esters being tested. Added Sections to conform to ISO 17025 standards. |
| 02/13/2019 | Cleaned up calculations, clarified how method is used for C16:1 Omega 7 (palmitoleic) analysis. |