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ORIGINAL SUBMISSION

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GENERALLY RECOGNIZED AS SAFE (GRAS) NOTIFICATION

HIOMEGA™ FLAXSEED OIL

POLAR FOODS, INC.

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1. GRAS EXEMPTION CLAIM

Polar Foods, Inc. through it agent Lillian Peterson, M.Sc., Golas R&D Consultants Inc., hereby notifies the Food and Drug Administration the use of HiOmega™ flaxseed oil described herein is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act because Polar Foods, Inc. has determined that such use is generally recognized as safe (GRAS).

[redacted]
Lillian Peterson, M.Sc.
Golas R&D Consultants, Inc.
Agent for Polar Foods, Inc.

May 20, 2008
Date

1.1 Name and Address of Notifier

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1.2 Common or usual name of GRAS substance

The common name of the substance that is the subject of this Generally Recognized as Safe (GRAS) notification is HiOmega™ flaxseed oil, a vegetable oil which is cold pressed from patented HiOmega™ flaxseed (*Linum usitatissimum*).

1.3 Levels of use in foods

Polar Foods, Inc. markets HiOmega™ flaxseed oil as a nutrient supplement (21 CFR 170.3(o)(20)). In accordance with good manufacturing practices, HiOmega™ flaxseed oil is intended for use as replacement for edible oils in numerous food categories resulting in an estimated dietary intake of less than 36 g HiOmega™ flaxseed oil/day or 25 g of ALA/day. The food categories proposed for addition of HiOmega™ flaxseed oil and respective addition levels are listed in Table 1. These levels are % reference amount customarily consumed (RACC) as defined in 21 CFR 101.12 and are suggested maximua intended to keep overall exposure below 36g/day. The intended use levels for HiOmega™ flaxseed oil as listed in Table 1 were established by scientific review of the published, peer reviewed scientific literature regarding the physiological effects of dietary flaxseed oil consumption, by estimating potential human exposure to ALA from the intended uses of HiOmega™ flaxseed oil, and by estimating the potential human exposure to EPA and DHA as a result of possible ALA metabolic conversion to EPA and

DHA. This method is certain to overestimate actual dietary exposure to flaxseed oil, as it assumes that all foods in the listed categories would contain HiOmega™ flaxseed oil.

1.4 Basis for GRAS determination

This GRAS notification of HiOmega™ flaxseed oil as safe and also GRAS under the Federal Food, Drug, and Cosmetic Act (the Act) for direct addition to foods as a nutrient supplement at specified levels (Table 1) based upon scientific procedures under 21 CFR 170.30(b).

1.5 Availability of Information

The data and information that serve as the basis for this GRAS notification will be sent to FDA upon request, or are available for the FDA's review and copying at reasonable times at the offices of Lillian Peterson, M.Sc., Golas R&D Consultants, Inc., agent of Polar Foods, Inc.

Table 1. Maximum levels of use of HiOmega™ oil in foods.

Category of Food [1]	RACC (g)[2]	ALA addition level (%)[3]	HiOmega™ Flaxseed Oil addition level (%)
Baked goods and baking mixes (1)	55	8	12
Beverages, alcoholic (2)	125*	1	1
Beverages and beverage bases, nonalcoholic (3)	312*	1	1
Cereals (4)	39	7	10
Cheeses (5)	50	8	12
Chewing gum (6)	3	5	7
Coffee and tea (7)	240	8	12
Condiments and relishes (8)	20	8	12
Confections and frostings (9)	33	8	12
Dairy product analogs (10)	23	8	12
Egg products (11)	110	8	12
Fats and oils (12)	16	20	29
Fish products (13)	81	8	12
Frozen dairy desserts (20)	240	8	12
Fruit and water ices (21)	85	8	12
Gelatins, puddings, and fillings (22)	120	1	2
Grain products and pastas (23)	80	4	5
Gravies and sauces (24)	30	8	12

Hard candy (25)	7	17	24
Jams and jellies, commercial (28)	15	12	17
Meat products (29)	33	8	12
Milk, whole and skim (30)	85	8	12
Milk products (31)	96	8	12
Nut and nut products (32)	25	8	12
Plant protein products (33)	98*	8	12
Poultry products (34)	85	5	7
Processed fruits and fruit juices (35)	39	1	2
Processed vegetables and vegetable juices (36)	107	1	2
Snack foods (37)	30	8	12
Soft candy (38)	28	7	10
Soups and soup mixes (40)	245	5	7
Sugar, white, granulated (41)	4	6	9
Sugar substitutes (42)	4	17	24
Sweet sauces, toppings and syrups (43)	45	8	12

1. Refers to the food category in 21 CFR 170.3 (n).
2. Average of Reference Amount Customarily Consumed (21 CFR 101.12) except for * categories which are calculated by averaging the USDA common measure amounts (USDA National Nutrient Database for Standard Reference, Release 19).
3. Assuming HiOmega™ flaxseed oil contains 70 % alpha linolenic acid (ALA), rounded to nearest whole number.

2. IDENTITY OF GRAS SUBSTANCE

2.1 Introduction

HiOmega™ flaxseed was developed from traditional flaxseed (linseed) by Dr. Edward Kenaschuck in a collaborative plant breeding program at the Morden Research Station, Agriculture and Agri-Food Canada, Morden, Manitoba, Canada and is described in United States Patent # 6,870,077 which is included by reference in its entirety herein. HiOmega™ is the trademark name for high linolenic acid flax seed, oil, and meal produced and marketed by Polar Foods Inc. (PFI) of Fisher Branch, Manitoba, Canada. HiOmega™ flaxseed, and thus HiOmega™ flaxseed oil, is not genetically modified. HiOmega™ flaxseed oil is naturally composed of a mixture of fatty acids in the form of triacylglycerides. The fatty acid moieties in HiOmega™ flaxseed are primarily 70 ± 3 % alpha-linolenic acid (ALA), 10 ± 2 % linoleic acid (LA), 12 ± 2 % oleic acid, 4 ± 2% stearic acid, and 4 ± 2% palmitic acid. HiOmega™ flaxseed oil is substantially similar to conventional flaxseed oil as shown in Table 2. It is also similar to other vegetable oils such as canola, corn, olive, peanut, safflower, soybean, sunflower and walnut oils (Table 2). Some of these oils and fatty acids such as canola oil (GRN 33), coconut oil, peanut oil, linoleic acid and oleic acid (LRSO/FASEB SCOGS-65, 1975) and hydrogenated soybean oil (LRSO/FASEB SCOGS-70, 1976) are already considered GRAS for nutrient or dietary additives. Glyceryl monostearate, glyceryl palmitostearate and glyceryl monooleate are also considered GRAS. The fatty acid profile of HiOmega™ flaxseed oil is also compared to edible fish oils (Table 3). HiOmega™ flaxseed oil is sold as a liquid oil.

Table 2. Lipid profile of Polar Foods Inc.'s HiOmega™ flaxseed oil and typical edible vegetable oils.

Lipids	Percent By Weight									
	HiOmega™	Flaxseed	Canola	Corn	Olive	Peanut	Safflower	Soybean	Sunflower	Walnut
Saturated	**6.72**	**9.4**	**7.1**	**12.95**	**13.81**	**16.9**	**6.20**	**14.9**	**9.78**	**9.1**
10:0	0	0	0	0	0	0	0	0	nl	0
12:0	0	0	0	0	0	0	0	0	nl	0
14:0	0	0	0	0.02	0	0.1	0	0.1	nl	0
15:0	0	nl	nl	nl	nl	nl	nl	nl	0.8	nl
16:0	3.97	5.3	4.0	10.58	11.29	9.5	4.29	9.8	3.68	7.0
17:0	0	nl	nl	0.07	0.02	nl	nl	nl	nl	nl
18:0	2.34	4.1	1.8	1.85	1.95	2.2	1.92	5.0	4.32	2.0
20:0	0.31	nl	0.7	0.43	0.41	1.4	nl	nl	nl	nl
22:0	0.1	nl	0.4	0	0.13	2.8	nl	nl	1	nl
24:0	0	nl	0.2	nl	0	0.9	nl	nl	nl	nl
Monounsat.	**10.89**	**20.2**	**58.9**	**27.58**	**72.96**	**46.2**	**14.36**	**43.0**	**83.59**	**22.8**
16:1 undiff.	0.025	0	0.2	0.11	1.26	0.1	0	0.4	nl	0.1
17.1	0.025	nl	nl	nl	0.13	nl	nl	nl	nl	nl
18:1 undiff.	10.74	20.2	56.1	27.33	71.27	44.8	14.36	42.5	82.63	22.2
20:1	0.025	0	1.7	0.13	0.31	1.3	0	0.0	0.96	0.4
22:1 undiff	0.075	0	0.6	0	0	0	0	0	nl	0
Polyunsat.	**82.27**	**66.0**	**29.6**	**54.68**	**10.52**	**32.0**	**74.62**	**37.6**	**3.80**	**63.3**
18:2 undiff.	10.83	12.7	20.3	53.52	9.76	32.0	74.62	34.9	3.61	52.9
18:3 undiff.	71.44	53.3	9.3	1.61	0.76	0	0	2.6	0.19	10.4
18:4	0	0	0	0	0	0	0	0	nl	0
20:4	0	0	0	0	0	0	0	0	nl	0
20:5 n-3	0	0	0	0	0	0	0	0	nl	0
22:5 n-3	0	0	0	0	0	0	0	0	nl	0
22:6 n-3	0	0	0	0	0	0	0	0	nl	0
Cholesterol	0	0	0	0	0	0	0	0	0	0
Phytosterols	nl	nl	nl	nl	0.22	0.21	nl	0.13	nl	0.18

Source of data:
1. **HiOmega™ : Average of 5 lots produced by Polar Foods, Inc.**
2. **Conventional flaxseed, canola, corn, sunflower (high oleic), safflower (70 % linoleic), soybean (hydrogenated), olive, peanut and walnut: USDA National Nutrient Database for Standard Reference, Release 19 (2006). Values rounded to 2 decimal places.**

nl: not listed

Table 3. Lipid Profile of Polar Foods Inc.'s HiOmega™ flaxseed oil and typical edible fish oils.

Lipids	Percent By Weight						
	HiOmega™	Cod Liver	Herring	Menhadren	Salmon	Sardine	18/12 TG
Saturated	**6.72**	**22.61**	**21.29**	**30.43**	**19.87**	**29.89**	**27.98**
10:0	0	nl	nl	nl	nl	nl	nl
12:0	0	nl	0.16	nl	nl	0.10	nl
14:0	0	3.57	7.17	7.96	3.28	6.53	7.42
15:0	0	nl	nl	15.15	nl	nl	nl
16:0	3.97	10.63	11.70	3.78	9.84	16.65	17.05
17:0	0	nl	nl	nl	nl	nl	nl
18:0	2.34	2.80	0.82	nl	4.25	3.89	3.51
20:0	0.31	nl	nl	nl	nl	nl	nl
22:0	0.1	nl	nl	nl	nl	nl	nl
24:0	0	nl	nl	nl	nl	nl	nl
Monounsat.	**10.89**	**46.71**	**56.56**	**26.69**	**29.04**	**33.84**	**21.06**
16:1 undiff.	0.025	8.31	9.64	10.48	4.82	7.51	8.46
17.1	0.025	nl	nl	nl	nl	nl	nl
18:1 undiff.	10.74	20.65	11.96	14.53	16.98	14.75	12.60
20:1	0.025	10.42	13.63	1.33	3.86	5.99	0
22:1 undiff	0.075	7.33	20.61	0.35	3.38	5.59	0
Polyunsat.	**82.27**	**22.54**	**15.60**	**34.20**	**40.32**	**31.87**	**40.91**
18:2 undiff.	10.83	0.94	1.15	2.15	1.54	2.01	1.47
18:3 undiff.	71.44	0.94	0.76	1.49	1.06	1.33	1.51
18:4	0	0.94	2.31	2.74	2.80	3.03	3.05
20:4	0	0.94	0.29	1.17	0.68	1.76	2.08
20:5 n-3	0	6.90	6.27	13.17	13.02	10.14	18.55
22:5 n-3	0	0.94	0.62	4.92	2.99	1.97	2.40
22:6 n-3	0	10.97	4.21	8.56	18.23	10.66	11.85
Cholesterol	0	0.57	0.77	0.52	0.49	0.71	nl
Phytosterols	nl	na	na	na	na	na	na

1. HiOmega™ : Average of 5 lots produced by Polar Foods, Inc.
2. Cod liver, herring, menhadren, salmon, sardine: USDA National Nutrient Database for Standard Reference, Release 19 (2006)
3. 18/12 TG: GRN # 138 Notification (2003). Values rounded to 2 decimal places. nl: not listed na: not applicable

2.2 Chemical name of the GRAS substance

A single chemical name is not assigned to HiOmega™ flaxseed oil. Similar to other vegetable oils, HiOmega™ flaxseed oil is a mixture of fatty acids, primarily in the form of triacylglycerides. The fatty acids are primarily alpha linolenic acid, linoleic acid, oleic acid, stearic acid and palmitic acid. Of the fatty acids present, alpha linolenic acid constitutes 68 – 73 %, linoleic acid constitutes 9 – 12 %, oleic acid constitutes 9 – 14 %, stearic acid constitutes 2 – 6 % and palmitic acid constitutes 3 – 6 %. Other components present in small quantities (1-2 %) include sterols, tocopherols, pigments and other minor constituents.

2.3 Chemical Abstract Service (CAS) registry number

Since HiOmega™ flaxseed oil that is the subject of this GRAS determination is a mixture of fatty acids, a Chemical Abstracts Service registry number has not been assigned (Table 4). However, a Chemical Abstracts Service registry number has been assigned to refined regular flaxseed oil also known as linseed oil (Table 4).

Table 4. Chemical Abstract Service (CAS) registry numbers and common names for fatty acids present in HiOmega™ flaxseed oil.

Fatty Acid	Common Name	CAS Registry Number
18:3 n-3	Linolenic Acid	028290-79-1
18:2 n-6	Linoleic Acid	000060-33-3
18:1	Oleic Acid	000112-80-1
18:0	Stearic Acid	000057-11-4
16:0	Palmitic Acid	000057-10-3
Linseed oil	Flaxseed Oil	8001-26-1

2.4 Empirical formula

A single molecular or structural formula does not exist for HiOmega™ flaxseed oil, the subject of this GRAS determination, because HiOmega™ flaxseed oil is a mixture of fatty acids. The molecular formulas for alpha linolenic acid, linoleic, oleic, stearic and palmitic acid, the major fatty acid components of HiOmega™ flaxseed oil, are described herein and listed in Table 5. The structural formulas for these fatty acids are shown in Figures 1 to 5 respectively.

Alpha-linolenic acid (ALA) is a polyunsaturated omega-3 fatty acid with the molecular formula $C_{18}H_{30}O_2$ and molar mass 278.43 g/mol. In physiological literature, alpha linolenic acid may be referred to as C18:3(n-3). The systematic chemical name for alpha linolenic acid is *all-cis*-9,12,15-octadecatrienoic acid (Table 5). The structual formula for ALA is shown in Figure 1.

Linoleic acid (LA) is polyunsaturated omega-6 fatty acid with the molecular formula $C_{18}H_{32}O_2$ and molar mass 280.43 g/mol. In physiological literature linoleic acid may be referred to as C18:2(n-6). The systematic chemical name for linoleic acid is *cis,cis*-octadeca-9,12-dienoic acid (Table 5). The structural formula for LA is shown in Figure 2.

Oleic acid is a monounsaturated fatty acid with the molecular formula $C_{18}H_{34}O_2$ and molar mass 282.45 g/mol. In physiological literature oleic acid may be referred to as C18:1(n-9). The systematic chemical name for oleic acid is *cis*-octadec-9-enoic acid (Table 5). The structural formula for oleic acid is shown in Figure 3.

Stearic acid is a saturated fatty acid with the molecular formula $C_{18}H_{36}O_2$ and molar mass 284.47 g/mol. In physiological literature stearic acid may be referred to as 18:0.

The systematic chemical name for stearic acid is octadecanoic acid (Table 5). The structural formula for stearic acid is shown in Figure 4.

Palmitic acid is a saturated fatty acid with the molecular formula $C_{16}H_{32}O_2$ and molar mass 256.42 g/mol. In physiological literature palmitic acid may be referred to as C16:0. The systematic chemical name for palmitic acid is hexadecanoic acid (Table 5). The structural formula for palmitic acid is shown in Figure 5.

Table 5. Molecular formulas for major fatty acid components of HiOmega™ flaxseed oil.

Common Name	Chemical Name	Molecular Formula
Alpha Linolenic Acid	$C_{18}H_{30}O_2$	*all-cis*-9,12,15-octadecatrienoic acid
Linoleic Acid	$C_{18}H_{32}O_2$	*cis, cis*-9,12-octadecadienoic acid
Oleic Acid	$C_{18}H_{34}O_2$	*cis*-octadec-9-enoic acid
Stearic Acid	$C_{18}H_{36}O_2$	octadecanoic acid
Palmitic Acid	$C_{16}H_{32}O_2$	hexadecanoic acid

2.5 Structural formulas

Figure 1. The structural formula of alpha linolenic acid showing physiological numbering (top) and chemical numbering (bottom) conventions

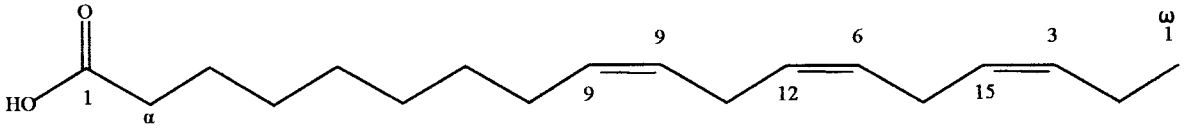


Figure 2. The structural formula of linoleic acid showing physiological numbering (top) and chemical numbering (bottom) conventions.

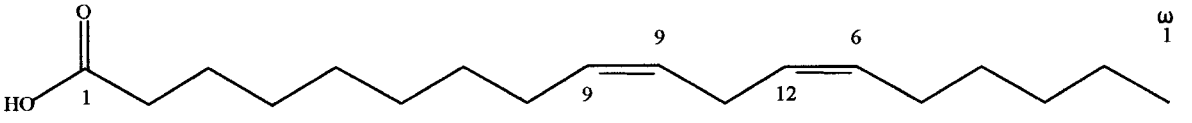


Figure 3. The structural formula of oleic acid showing physiological numbering (top) and chemical numbering (bottom) conventions.

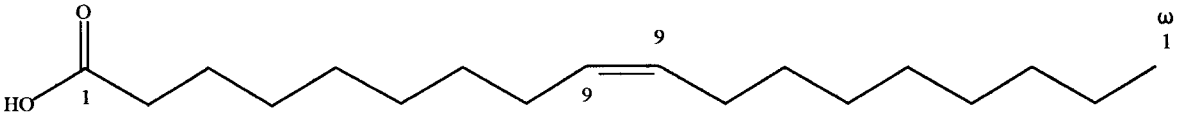


Figure 4. The structural formula of stearic acid.

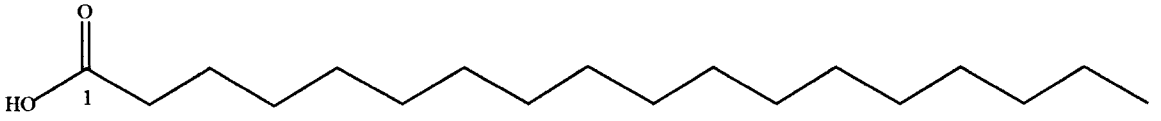
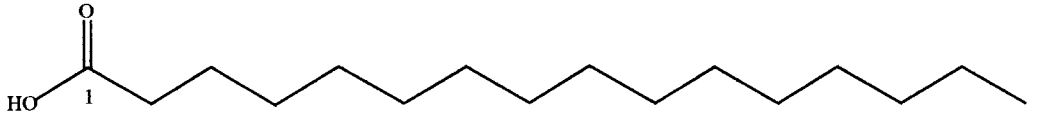


Figure 5. The structural formula of palmitic acid.



2.6. Specifications for food grade material

Polar Foods, Inc. has developed specifications for HiOmega™ flaxseed oil to demonstrate that it is food grade. The oil production facility is Kosher inspected and approved by OK Kosher. The oil production facility is Organic inspected by Letis and meets NOP standards. The oil production operation is in compliance with the Good Manufacturing Production Program. The oil production facility completed a Hazard Analysis and Critical Control Point (HAACP) Program. All HiOmega™ flaxseed oil produced has been extracted and purified by the processes described, or similar processes' using good manufacturing practice, and is Certified Kosher.

These specifications are listed below.

• Description:	HiOmega™ flaxseed oil is a mixture of fatty acids mostly in the form of triglycerides resulting from cold pressing and filtering HiOmega™ flaxseed.
• Appearance:	a golden yellow oil free of foreign matter
• Odor:	characteristic of flax oil (i.e., a natural vegetable oil scent without paint tones)
• ALA (C18:3)n3 content:	68 – 73 %
• LA (C18:2)n6 content:	9 – 12 %
• Oleic acid (C18:1) content:	9 – 14 %
• Stearic acid (C18:0) content:	2 - 6 %
• Palmetic acid (C16:0) content:	3 - 6 %
• Free Fatty Acid:	NMT 1 %
• Peroxide Value:	NMT 10 meq/kg
• Arsenic:	NMT 0.2 ppm
• Lead:	NMT 0.1 ppm
• Cadmium:	NMT 0.2 ppm
• Mercury:	NMT 0.1 ppm
• Cyanoglucosides:	NMT 2 ppm
• Viscosity:	40 centipoise

2.7. Quantitative compositions

Conformance with the above specifications is shown in the following tables (Tables 6 - 11). Results are from independent third party laboratory tests on several lots of HiOmega™ flaxseed oil.

Table 6. Batch analysis of fatty acids of five lots of Polar Foods, Inc.'s HiOmega™ flaxseed oil.

Parameter	Spec.	Lot # 2303/5	Lot # 733	Lot # 1210/47	Lot # 1210	Lot # 1210/2
Palmitic C16:0 (%)	3 - 6	3.88	3.50	4.30	4.18	4.20
Stearic C18:0 (%)	2 - 6	2.25	2.20	2.50	2.45	2.40
Oleic C18:1 (%)	9 - 14	11.24	10.00	11.40	9.91	10.30
Linoleic C18:2 (%)	9 - 12	11.43	9.50	11.60	10.67	10.80
α-Linolenic C18:3 (%)	68 - 73	70.66	73.80	69.60	72.12	71.70
C20:0 (%)	0.1 - 0.4	0.35	0.30	0.30	0.11	0.30
All other fatty acids	< 1.0	0.19	0.70	0.30	0.56	0.30

Table 7. Batch analysis of yeast, E.coli, mold and standard plate count of five lots of Polar Foods, Inc.'s HiOmega™ flaxseed oil.

Parameter	Specification	Lot # 1210/24	Lot # 1210	Lot # 1311/2	Lot # 384	Lot # 1210/27
Yeast(CFU/g)	NMT 300	0.00	0.00	0.00	<10	0.00
E. coli (CFU/g)	Negative	0.00	0.00	0.00	n/a	0.00
Mold (CFU/g)	NMT 300	<25	30	50	<10	<25
Standard Plate Count (CFU/g)	NMT 300	100.00	200.00	270.00	30.00	80.00

Table 8. Batch analysis of arsenic, lead, cadmium, mercury of three lots of Polar Foods, Inc.'s HiOmega™ flaxseed oil.

Parameter	Specification	Lot #1010/06	Lot # 1211/19	Lot # 1311/2
Arsenic (ppm)	NMT 0.2	<0.10	<0.10	<0.10
Lead (ppm)	NMT 0.1	<0.05	<0.05	<0.05
Cadmium (ppm)	NMT 0.2	<0.05	<0.05	<0.05
Mercury (ppm)	NMT 0.1	<0.03	<0.03	<0.03

Table 9. Batch analysis of peroxide value of five lots of Polar Foods, Inc. HiOmega™ flaxseed oil.						
Parameter	**Specification**	**Lot # 1210/4**	**Lot # 1210**	**Lot # 1508**	**Lot # 1510**	**Lot # 1607/3**
Peroxide value (meq/kg)	NMT 10	0.00	0.10	2.00	2.00	2.00

Table 10. Batch Analysis of free fatty acid of five lots of Polar Foods, Inc. HiOmega™ flaxseed oil.						
Parameter	**Specification**	**Lot # 1508**	**Lot # 1510**	**Lot # 1607/3**	**Lot # 384/1**	**Lot # 6993**
Free Fatty Acid (%)	NMT 1	0.50	0.40	0.30	0.25	0.63

Table 11. Testing methods for the idenfication and characterization of HiOmega™ flaxseed oil.	
Parameter	Testing Method
Fatty acid profile	AOCS Ce 1c-89
Sterols	AOCS Ch 6-91
Tocopherols	AOCS Ce 8-89
Iodine value	AOCS Cd 1d-92
Viscosity	AOCS Ja 11-87
Specific gravity	AOCS To 1a-64
Color	Gardner Scale
Peroxide value	AOCS Cd 8-53
Free fatty acids	AOCS Ca 5a-40
Phosphorus	AOCS Ca 12-55

2.8. Pesticide listing

No pesticides were detected by independent laboratory pesticide scans for pesticide residues, dioxins, furans, PCBs and PAHs.

2.9. Cyanoglucosides

No cyanoglucosides were detected by independent laboratory test.

2.10. Manufacturing process

Producing HiOmega™ flaxseed oil for direct addition to food involves the following steps four steps which are described below and shown as schematics in Figure 6 and Figure 7.

2.10.1. Raw material

HiOmega™ flaxseed was derived from traditional flax (*Linum usitatissimum*), which is widely adapted to temperate climates. The present growing areas include Argentina, Ethiopia, Canada, India, China, the USA, and the former Soviet Union. Total world production of flax was 2.15 million tons in 1993/94. Flax has been cultivated in various parts of the world for millennia. The influence of environment on the fatty acid composition (degree of unsaturation) of oilseed is well known. For example flaxseed oil extracted from flaxseed grown in cooler, more northerly parts of the world are higher in alpha linolenic acid than that grown in more southerly parts (Dillman et al. 1943).

In Canada, HiOmega™ flaxseed is grown commercially under identity preserved contract basis. Yields of HiOmega™ flaxseed are similar to those of the best adapted flax cultivars. Height is slightly shorter and seed size slightly smaller than that of other flax cultivars such as Mcduff, Hanly, or Norlin. Maturity and lodging resistance is comparable to Norlin. Development programs continue for HiOmega™ flaxseed in Saskatoon, Saskatchewan at the National Research Council and at Dr. Ed Kenaschuk's research facility in Morden, Manitoba. Lines from the development efforts have been

grown across western Canada. Furthermore, commercial crops of HiOmega™ have been harvested in Southern California and Chile.

A collaborative effort with Dr. Kenaschuck at the Agriculture Canada Morden Research Station commenced in 1989, and by 1996 selection for increased levels of linolenic acid levels produced lines containing as much as 67%. In 2001, the first lines were provisionally filed for patent in the United States Patent and Trademark Office (USPTO). The current lines are protected by USPTO number 6,870,077 included herein by reference (Kenaschuk 2005). Recent further development of these lines produced flax cultivars with linolenic acid levels of 74 %, 78 % & 86 %.

Non genetically modified high linolenic flax was developed by conventional plant breeding (Fig. 6) i.e. hybridization using cultivars, breeding lines and accessions, and selections for high linolenic acid content in segregating populations from crosses, and the AAFC Morden Research Centre, Morden, MB. The development of high linolenic flax involved 6 crosses. In each of the crosses, the F2 and subsequent generations of crosses were advanced to the F7 generation using the pedigree method of breeding. Selection for high linolenic was initiated in the F2 generation. F2 plants were analyzed on a half seed basis by gas-liquid chromatography of the fatty acid esters. Using the remainder of the seed, the F3 generation of high linolenic genotypes was grown in the greenhouse. Single plant selections were made in the F4 generation on basis of linolenic content. The F5 generation of selected lines was grown in a winter nursery. F6 lines were selected for high linolenic content at Morden, and seed harvested in bulk. High linolenic flax was evaluated in replicated trials conducted in 1998, 1999, and 2000, and in field trials conducted in 1998 and 1999.

Figure 6. Schematic of plant breeding of HiOmega™ flaxseed from conventional flaxseed.

Cross

F1-greenhouse

F2-field; high linolenic genotypes selected by ½ seed analysis

F3-greenhouse

F4-field; plants selected for high linolenic content

F5-winter nursery

F6-field; lines selected for high linolenic and seed harvested in bulk

F7-seed increase in winter nursery

F8-replicated trials in Manitoba

F9-replicated trials in Manitoba

F10-field trials in Alberta and Manitoba

2.10.2. Mechanical cold pressing

The technical aspects of extracting oil from oil bearing seeds are well known (Wiesenborn et al. 2005b). HiOmega™ flaxseed oil, the subject of this notification is mechanically removed from HiOmega™ flaxseed by expeller pressing below 122° F (50° C). The flaxseed oil is produced in an all natural physical process. No hexanes, solvents or chemicals are used to extract the flaxseed oil.

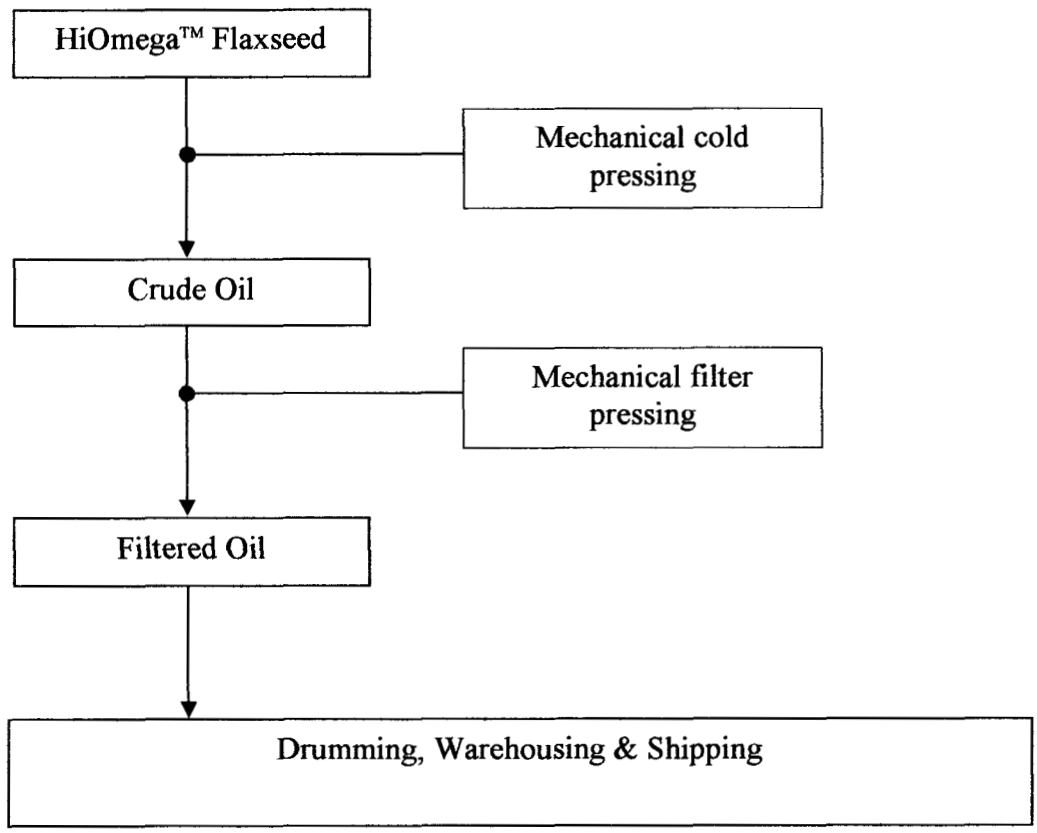
2.10.3. Mechanical filter pressing.

The HiOmega™ flaxseed oil is mechanically filter pressed to remove impurities.

2.10.4. Drumming, Warehousing and Shipping.

HiOmega™ flaxseed oil is packaged in bulk containers flushed with inert argon gas. Bulk containers may include drums, totes, pails or other food grade, food safe containers. Packaged HiOmega™ flaxseed oil is warehoused and shipped to fill customer orders.

Figure 7. Schematic of HiOmega™ flaxseed oil production.



3. USE OF GRAS SUBSTANCE

3.1. Date when use began

As described under the Raw Material section 2.10.1, HiOmega™ flaxseed has been derived from regular flaxseed which has been in use in foods for millennia.

3.2. Information and reports on past uses in food

HiOmega™ flaxseed oil should be considered a food ingredient. It is not a frying oil although HiOmega™ can be used as an additive in margarine and shortening. For example, flaxseed oil is used in Smart Balance Margarine handled by GFA Brands Inc. (www.smartbalance.com). HiOmega™ flaxseed oil is a functional ingredient in food manufacture of baked goods such as starches, proteins, dairy products, beverages, bars, salad oil, seasonings and sauces, cosmetic oils and pharmaceuticals, pet food. The packaging of the food product should be inert gas flushed, sealed, shelf life of one year, or temperature controlled or temperature controlled in a refrigerator after opening. Most of these applications are using traditional ground flaxseed and many for example are using traditional flaxseed oil. For example, interalia, HiOmega™ flaxeed oil is added in butter and cottage cheese by Farmers All Natural Creamery in Wellmann Iowa. Flaxseed oil is added to milk by Natrel (www.natrel.ca) of Quebec, Canada and distributed as Omega 3™ milk beverage. HiOmega™ flaxseed oil should be considered a substitute for other vegetable oils in food products which often contain mixtures of vegetable oils yielding the most desirable end products. Price, functionality and availability will

determine the extent of use of HiOmega™ flaxseed oil in various applications. The functions of HiOmega™ flaxseed oil in food will be identical to those of regular flaxseed/oil for which it substitutes. HiOmega™ flaxseed oil will provide less saturated fat in the diet, more unsaturated and polyunsaturated fat and a more beneficial omega 6:omega 3 ratio for a more nutritionally balanced diet.

3.3. Methods for detecting the GRAS substance in food

The concentration of HiOmega™ flaxseed oil in a food product, if present as the sole fat component or as a known proportion of the total fat component can be estimated by the determination of crude fat (AOCS BA3-38 or other similar method).

4. SUMMARY OF BASIS OF GRAS NOTIFICATION

4.1. Scientific Literature Review of Safety Data

4.1.1 Introduction

For the purposes of this GRAS notification the term 'flaxseed oil' is used since the term flaxseed oil is used interchangeably with the terms 'linseed oil' and 'flaxoil' within the literature.

A thorough literature review was conducted to assess the safety of dietary flaxseed oil. The effects of flaxseed oil in human and animal studies were examined for safety and adverse effects. The physiology reviewed includes absorption, digestion, metabolism and excretion, effects on fatty acid profiles, bleeding time, glycemic control, cholesterol, triglycerides, blood pressure, eicosanoids, cardiovascular health, immune system, cancer, body weight, bone health, kidneys, liver, reproduction, eyes, skin, mental health, lungs, antibiotic, enzyme activity, blood, minerals and vitamins, miscellaneous effects and oxidative stability. No serious, clinically relevant adverse effects due to dietary flaxseed oil supplementation in humans were indicated.

4.1.2 Adverse Effects

Several reviews (Morris 2007, Thompson et al. 2003, Burdge 2006, Morris 2003, Singer 1992, Wijendran et al. 2004, Monahan 2007, Zollner 1986, Simopoulos 1999, Simopoulos 1999b, Simopoulos 2000, Wendland et al. 2006, Basch et al. 2007) and

clinical case reports (Galland 1986, Rudin 1981, Rudin 1982) indicate the beneficial effects of dietary intake of flaxseed oil and/or ALA with little or no adverse side effects. Several studies explicitly stated that no adverse effects occurred or that flaxseed oil supplements were well tolerated (Natvig et al. 1968, Freese et al. 1997, Freese et al.1997b, Wilkinson et al. 2005, Nelson et al. 2007, Kestin et al. 1990, Henry et al. 1990) and some studies indicated minor side effects such as slight nausea or mild diarrhea which were not clinically relevant (Borchgrevink et al. 1965, Wang et al. 2006). Several animal studies indicated no effect, beneficial effects or protective effects of dietary flaxseed oil supplementation (Brändle et al.1997, Takeuchi et al. 2000, Takeuchi et al. 2001b, Bhatia et al. 2006, Bhatia et al. 2007, McClain et al. 1985). Details of specific examples of human and animal studies follow.

No adverse effects were noted for volunteers consuming dietary supplementation with flaxseed oil (10 ml oil/day, 5.5 ml ALA/day, n6:n3 ratio 0.27) (Natvig et al. 1968). Dietary supplementation with encapsulated flaxseed oil was well tolerated and no major gastric-side effects were reported for healthy volunteers consuming dietary encapsulated flaxseed oil supplements (6.21 g/day ALA, 11.9 g oil/day, n6:n3 ratio 0.27 on average) (Freese et al. 1997b). Sachets of dietary oil were well tolerated and successfully incorporated into cooked foods including pasta sauces, salad dressings and milk shakes for normal, healthy volunteers expressing an atherogenic lipoprotein phenotype consuming dietary supplements of flaxseed oil (30 g oil/day, 15 g ALA per day, n-6:n3 ratio <1) (Wilkinson et al. 2005). No major gastric side effects were reported and no subjects left the study due to supplementation with encapsulated flaxseed oil taken with meals (1 g oil/capsule, 55 % ALA, 5.9 g ALA/day, n6:n3 ration 0.27) (Freese et al.

1997). No adverse effects were noted for overweight but otherwise healthy volunteers consuming a diet supplemented with flaxseed oil capsules (ALA 5 % of energy intake, 57 % ALA, n6:n3 ratio 0.32) (Nelson et al. 2007). Dietary supplements of flaxseed oil (9.2 g ALA per serving, n6:n3 ratio 0.48) emulsified in low fat milk did not affect a complete hematological screen, plasma electrolytes, renal and liver function tests for normotensive, mildly hypercholesterolemic volunteers (Kestin et al.1990). Mild side effects were noted with subjects supplemented with 20 or 30 ml of oil and included slight nausea or dyspepsia and mild diarrhea (Borchgrevink et al. 1965). However, these mild side effects are seen with consumption of larger quantities of oil, quantities far beyond the intended uses of HiOmega™ flaxseed oil as specified in Table1. In a review by Wang et al. (2006) it is noted that any adverse effects due to ALA supplementation were 'minor and bothersome but not clinically relevant.'

No adverse effects were noted for rats consuming a diet supplemented with flaxseed oil (8% oil, 45.52 % ALA, n6:n3 ratio 0.47) added to rations (Moore et al. 1991). No toxic effects were noted for mice administered flaxseed oil (0.1 ml oil/kg body weight) orally (Bhatia et al. 2006). No adverse effects were noted for horses fed an 8 % flaxseed oil-enriched pelleted ration (Henry et al. 1990). Few metabolic changes attributable to diet were noted for stressed rats consuming a basal diet supplemented with flaxseed oil (20 % oil, 55.5 % ALA) (Takeuchi et al. 2000). Body changes due to stress were not affected by flaxseed oil supplementation for stressed male rats consuming a basal diet supplemented with flaxseed oil (20 % oil, 55.5 % ALA, n6:n3 ratio 0.28) (Takeuchi et al. 2001b).

Protective effects of flaxseed oil were noted in several animal studies (Brändle et al. 1997, Bhatia et al. 2006, Bhatia et al. 2007, McClain et al.1985, Turek et al.1993).

Life span was increased for hypertensive rats fed flaxseed oil (2.5 % oil, 53.3 % ALA, n6:n3 ratio 0.39) added to a standard diet (Brändle et al. 1997).

Cyclophosphamide-induced reduction in glutathione, glutathione peroxidase and alkaline phosphatase levels and cyclophosphamide-induced increases in acid phosphatase activity and oxidized glutathione were prevented for mice administered flaxseed oil (0.1 ml/kg body weight) orally (Bhatia et al. 2006). These results indicated flaxseed oil prevented radiomimetic cyclophosphamide-induced oxidative stress in mouse brain. Survival rate and body weight gain were increased, radiation induced increases in lipid peroxidation, aspartate aminotransferase, alanine aminotransferase and acid phosphatase were significantly reduced and radiation induced decreases in glutathione and alkaline phosphatase activities were reduced for mice receiving whole body radiation and administered flaxseed oil orally (Bhatia et al. 2007).

Incidence of fetal cleft palate was reduced and hexobarbital sleeping time returned to control levels for rats consuming a standard diet with flaxseed oil (5 % oil) in place of corn oil (McClain et al.1985).

Morbidity was reduced and diarrhea did not occur however stomach, jejunal and ileal ulcer incidence was increased for male rats consuming a nutritionally balanced diet supplemented with flaxseed oil (12.5 % oil, 47 % ALA) as compared to a diet supplemented with corn oil (Turek et al.1993). In a review of possible deleterious effects of very high levels of ALA supplementation with perilla oil which typically contains 50 – 60 % ALA (Okuyama et al. 1997), it is suggested that the formation of ulcers is similar to

those produced by prolonged administration of aspirin and indomethacin. An n6:n3 ratio of 1 was considered safe, however it is noted that this ratio is difficult to obtain within a typical diet and there would be very few human cases where a low n6:n3 ratio would induce stomach ulcers (Okuyama et al. 1997).

In conclusion, no serious adverse effects were noted in human studies of dietary supplementation of flaxseed oil at levels as high as 30 g flaxseed oil/day equivalent to 15 g ALA/day. These levels are much higher than the adequate daily intake of ALA set by the FDA (1.6 g ALA/day for men and 1.1 g ALA/day for women). Similarly, no serious adverse effects of flaxseed oil supplementation were noted in animal studies (Moore et al. 1991, Bhatia et al. 2006), rather, flaxseed oil appears to exert protective effects such as increased life span (Brändle et al. 1997), reduced morbidity (Turek et al.1993), reduced incidence of cleft palate (McClain et al.1985) and protection from radiation (Bhatia et al. 2007). One animal study indicated an increase in ulcer incidence (Turek et al. 1993) at a level of ALA intake and n6:n3 ratio unlikely to be achieved in a typical human diet. Therefore, Polar Foods, Inc. has determined that adverse effects will not result from HiOmega™ flaxseed oil dietary supplementation at its intended uses as set out in Table 1.

4.1.3 Absorption, Distribution, Metabolism and Excretion

Absorption

There is evidence that ALA is efficiently absorbed by the digestive system (Saunders et al. 1988, Burdge 2006). Healthy volunteers with ileostomies consuming flaxseed oil (5 x 100 g doses of flaxseed oil, 44 % ALA) as part of a liquid meal absorbed approximately 98 % of ALA as triglyceride in the small intestine (Saunders et al. 1988).

Approximately 96 % of labeled ALA (750 mg ALA) was absorbed by one individual i.e. excretion of labeled ALA was approximately 4 % (Burdge 2006).

Distribution, Metabolism and Excretion

Dietary ALA may be utilized in the following ways:

a) Energy production i.e. β-oxidation, with shunting of acetate to complete oxidation to CO_2. It is estimated that 20 – 33 % of dietary ALA is used in this manner (Sinclair et al. 2002, Brenna 2002, Vermunt et al. 2000, DeLany et al. 2000, Bretillon et al. 2001, Burdge et al. 2002b). In humans, ALA does not appear to be preferentially used for energy production rather the use of ALA depends on the energy needs of the organism (Burdge et al. 2002b).

b) Biosynthesis of monounsaturated fatty acids, saturated fatty acids and cholesterol (Cunnane 2004, Brenna 2002, Sinclair et al. 2002).

c) Incorporation into phospholipids and cell membranes (Table 12 of this GRAS notification, Fu et al. 2000).

d) Conversion to long chain polyunsaturated fatty acids (n-3) such as EPA and DHA (Table 12, Fig. 8). For rats, 1.4 % of ALA is converted to long chain PUFA (EPA and DHA) (Cunnane et al. 1997). Although estimates for conversion of ALA to EPA range from 0.2 – 21 % and 0 – 9 % for conversion of ALA to DHA (DeFilippis et al. 2006), a commonly accepted range is 8 – 12 % conversion of ALA to EPA+DHA (Burdge et al. 2005b). The variation in these estimates may be due to a number of factors which are discussed below.

e) Transport to skin and fur (Fu et al. 2001, Fu et al. 2000b, Fiennes et al. 1973). Transport of ALA to the skin and fur may account for 46 % of labeled ALA in guinea pigs (Fu et al. 2000b).

f) Storage in adipose tissue (Fu et al. 2001, Hill et al. 2000). For rats, 10.9% of ALA may accumulate in fatty tissue (Cunnane et al. 1997).

g) The remainder of ALA is excreted in the stool. It is estimated that 2.2 - 4 % of ALA is excreted (Burdge 2006, Saunders et al. 1988, Cunnane et al. 1997).

Conversion of ALA to long chain PUFA EPA and DHA

ALA is converted to long chain polyunsaturated fatty acids such as EPA and DHA (Table 12, Fig. 8, Burdge et al. 2002b, Emken et al. 1994, Rosell et al. 2005, Sanders 1999, Sanders et al. 1987, Su et al. 2000, Organisciak et al. 1986, Stinson et al. 1991, Wang et al. 1992, Barceló-Coblijn et al. 2005, Wiegand et al. 1995, Goyens et al. 2006b) although estimated amount of ALA converted are variable.

Dietary intake studies are consistent with *in vitro* studies of ALA with conversion to lcPUFA. *In vitro* studies indicate conversion of ALA to EPA is linear i.e. increasing ALA levels result in proportionally higher EPA levels (Portolesi et al. 2007). Incorporation of ALA itself into membrane phospholipids is also linearly related to ALA levels. However, DHA levels in membrane lipids appear to be more tightly regulated. For example, conversion of ALA to DHA saturates with increasing ALA levels. Similarly, DHA levels saturate with increasing DHA supplementation. However, conversion of ALA to DHA may be more likely to occur if DHA levels are low (Portolesi

et al. 2007). Over time, ALA is converted to DHA to maintain initial DHA levels (Portolesi et al. 2007).

Factors which affect conversion

A number of factors may influence ALA conversion to lcPUFA including gender, background diet (trans fat, saturated fat, LA, EPA and DHA levels) and subsequent competition for the Δ6-desaturase enzyme, timing and 'location' of measurement.

Gender

Women may have a greater ability to convert ALA to lcPUFA possibly due to estrogen stimulation of the n3 metabolic pathway (Burdge 2006, Burdge 2004, Burdge et al. 2005, Burdge et al. 2005b, Burdge et al. 2002, Burdge et al. 2002b).

Background diet

The background diet may affect ALA conversion to lcPUFA. For example, trans fatty acid intake may inhibit Δ6 desaturase activity (Elias 2001). As reviewed by Gerster (1998), the amount of saturated fat in the diet may also affect the conversion rate. Dietary intake of EPA and DHA may inhibit ALA conversion to lcPUFA (Emken et al. 1999, Vermunt et al. 2000, Burdge et al. 2003).

Increased levels of LA may limit conversion of ALA to long chain polyunsaturated fatty acids (Burdge et al. 2005). The Δ6-desaturase enzyme is used in multiple steps in the n3 and n6 fatty acid metabolic pathways (Fig. 8). ALA competes with linoleic acid for this enzyme although ALA is the preferred substrate (Sanders et al.

1987). Conversely, formation of n6 lcPUFA is decreased and conversion of ALA to EPA is increased as the n6:n3 ratio is decreased for male volunteers consuming a diet supplemented with flaxseed oil (18 g ALA/day, 56 % ALA, n6:n3 ratio of diet ~ 0.5) added to cooked foods such as pasta sauces, salad dressings, and milkshakes (Hussein et al. 2005). Similarly, conversion of ALA to EPA (but not DHA) is increased by decreasing the ratio of n6:n3 fatty acids (Pan et al. 1993). ALA conversion to DHA may be optimized by lowering the LA to ALA ratio (Blank 2002) although other studies have shown no effect (Pan et al. 1993). However, some studies indicate that the total amount of dietary intake of ALA rather than the linoleic to α linolenic acid ratio may influence ALA conversion to lcPUFA (Goyens et al. 2006c).

Competition within metabolic pathways for Δ6-desaturase enzyme

As mentioned above, more than one substrate competes for the Δ6-desaturase enzyme in the n3 metabolic pathway (Fig. 8). Through competitive inhibition of Δ6-desaturase, tetracosapentaenoic acid (24:5n3) may limit conversion of ALA to DHA (Portolesi et al. 2007). This and other mechanisms may tightly regulate membrane DHA levels.

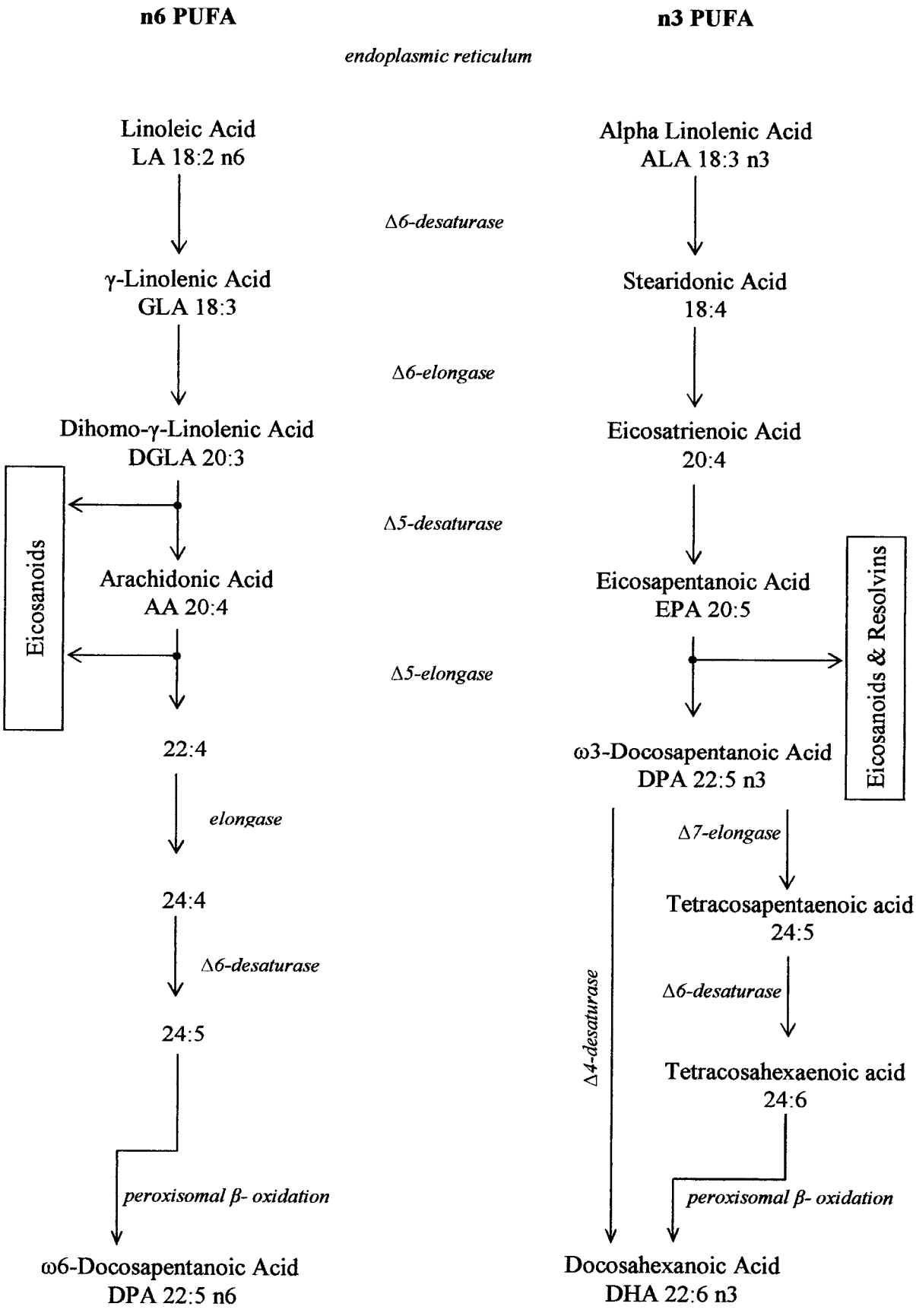
Tissue Type

The amount of ALA converted to lcPUFA may depend on where the measurement was taken (Table 12). For example, DHA synthesis in brain tissue is greater than DHA synthesis in liver tissue (Sanders et al. 1987).

Timing

Fatty acid levels measured in adipose tissue indicate long term dietary intake, however, measurement of fatty acid levels in membrane phospholipids reflect short term intake and these levels may be sensitive to time of measurement. For example, incorporation of ALA into membrane phospholipids was time dependent with greatest levels of ALA noted at 6 hours (Portolesi et al. 2007).

Fig. 8. Metabolism of ALA. Metabolic pathways for n6 and n3 polyunsaturated fatty acid adapted from Cunnane (2004), Arterburn et al. (2006), Gerster (1998), Lunn et al. (2006) and Burdge et al. (2005b).


n6 PUFA
n3 PUFA
endoplasmic reticulum
Linoleic Acid
LA 18:2 n6
Δ6-desaturase
γ-Linolenic Acid
GLA 18:3
Δ6-elongase
Dihomo-γ-Linolenic Acid
DGLA 20:3
Eicosanoids
Δ5-desaturase
Arachidonic Acid
AA 20:4
Δ5-elongase
22:4
elongase
24:4
Δ6-desaturase
24:5
peroxisomal β- oxidation
ω6-Docosapentanoic Acid
DPA 22:5 n6
Alpha Linolenic Acid
ALA 18:3 n3
Stearidonic Acid
18:4
Eicosatrienoic Acid
20:4
Eicosapentanoic Acid
EPA 20:5
Eicosanoids & Resolvins
ω3-Docosapentanoic Acid
DPA 22:5 n3
Δ7-elongase
Tetracosapentaenoic acid
24:5
Δ6-desaturase
Tetracosahexaenoic acid
24:6
Δ4-desaturase
peroxisomal β- oxidation
Docosahexanoic Acid
DHA 22:6 n3

4.1.4 Effect on Fatty Acid Profiles

Fatty acid profiles tend to change as a result of flaxseed oil supplementation (Table 12). Generally, if an effect is noted, there is an increase in ALA, EPA and DHA levels and a decrease in AA levels. Some anomalous results are noted such as a decrease in ALA (see Table 12. ref 57b) and EPA (see Table 12. ref 84a) levels. Since EPA and DHA levels may increase following flaxseed oil supplementation, the specific safety issues identified by the FDA for EPA and DHA were examined herein, not only in the context of dietary ALA consumption, but also in the context of possible conversion of ALA to EPA and DHA. These safety issues include a) increased bleeding time b) reduced glycemic control for diabetics c) increased levels of low-density lipoprotein (LDL) cholesterol among diabetics and hyperglycemics d) immunosuppressive effects (GRN # 138).

4.1.5 Bleeding Time

Bleeding time depends upon primary hemostasis (formation of the primary platelet plug) and mainly platelet function (Freese et al. 1997). Bleeding time for normal, healthy humans not on any medications is between 1 and 9 minutes (NIH, retrieved online 2007). Flaxseed oil supplementation does not increase bleeding times for humans beyond the normal range (Freese et al. 1997, Borchgrevink et al.1965, Kelley et al. 1993). Dietary supplementation with encapsulated flaxseed oil (~ 10.7 g oil/day, 55 % ALA, an average of 5.9 g ALA/day, n6:n3 ratio 0.27) increased bleeding time for healthy volunteers from 5.7 ± 1.6 minutes to 6.9 ± 2.4 minutes (mean ± SD) (Freese et al. 1997),

however this is within the range of normal bleeding times. Dietary supplementation with 10 to 30 ml of flaxseed oil per day did not affect bleeding times or platelet adhesion in adult males with a diagnosis of recent or impending myocardial infarction (15 ml ALA/day, 50 % ALA, n6:n3 ratio 0.34) (Borchgrevink et al. 1965). Bleeding times, prothrombin time, and partial prothrombin time were not affected for healthy volunteers consuming a diet of natural foods supplemented with flaxseed oil (31.7 g oil/day, 21.28 % ALA, 6.7 g ALA/day, n6:n3 ratio 0.72) which was cold mixed with yogurts, salads, sandwich spreads and vegetables (Kelley et al. 1993).

Bleeding time and coagulation is a result of a complex cascade of events. Several human and animal studies show that flaxseed oil has no effect of the components of this cascade (Kelley et al. 1993, Allman et al. 1995, Borchgrevink et al. 1965, Li et al. 1999, Schwab et al. 2006, Wilkinson et al. 2005, Freese et al.1997, Freese et al. 1997b, Blix et al. 1965). Flaxseed oil may have beneficial, normalizing effects on platelet adhesiveness in disease conditions (Owren et al.1965, Owren et al.1964). In these studies, patients with diabetes and coronary disease, consuming 20 ml of flaxseed oil per day reduced abnormally high platelet adhesiveness to normal levels (Owren et al.1965, Owren et al. 1964).

A human study has shown that collagen but not ADP induced platelet aggregation may be reduced (Allman et al. 1995) whereas other human studies have shown no effect on platelet aggregation (Li et al.1999, Freese et al. 1997, Freese et al.1997b, Wensing et al. 1999). Some human studies have shown plasma activity of plasminogen activator inhibitor I (PAI-1) may be increased (Wilkinson et al. 2005, Tohgi et al. 2004) whereas other human studies have shown no effect on PAI-1 activity (Schwab et al. 2006, Freese

et al.1997b). Some animal studies have shown that flaxseed oil consumption may reduce collagen and/or ADP-induced platelet aggregation (Bolton-Smith et al. 1984, Ramaprasad et al. 2005, Vas Dias et al. 1982). Details of specific examples of human and animal studies follow.

Collagen induced platelet aggregation was reduced whereas ADP induced platelet aggregation was not affected for healthy male volunteers consuming flaxseed oil (40 g oil/day, approximately 57 % ALA, n6:n3 ratio 0.28) with meals as compared to prior to flaxseed oil supplementation (Allman et al. 1995). In either fasting or post-prandial blood samples, dietary supplementation with encapsulated flaxseed oil (11.9 g flaxseed oil/day, 6.21 g ALA/day, n6:n3 ratio 0.27 on average) did not affect coagulation Factor VII (FVII:C), PAI-1 nor platelet aggregation in response to ADP or collagen for healthy volunteers as compared to pre-treatment or fish oil supplementation (Freese et al.1997b). No significant within treatment (10.7 g flaxseed oil/day, 5.9 g/day ALA, n6:n3 ratio 0.27 on average) or between treatment effects (as compared to fish oil supplementation) were noted on collagen or I-BOP induced platelet aggregation for healthy volunteers consuming dietary encapsulated flaxseed oil supplements (Freese et al. 1997). In the same study, flaxseed oil (10.7 g flaxseed oil/day, 5.9 g/day ALA, n6:n3 ratio 0.27 on average) did not affect ADP-induced platelet aggregation (Freese et al. 1997). For healthy, vegetarian volunteers, replacement of dietary fat with flaxseed oil (15.4 g ALA per day, n6:n3 ratio 1.0) and flaxseed oil based margarine did not affect *in vitro* agonist-induced whole blood platelet aggregation, full blood indices (white blood cell, red blood cell, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count and mean platelet volume) or

hemostatic factors (fibrinogen, Factor VII, prothrombin time, activated partial thromboplastin time, antithrombin III and plasminogen) (Li et al.1999).

Plasma activity of PAI-1 was increased whereas plasma fibrinogen and Factor VIIc were not affected for normal, healthy volunteers expressing an atherogenic lipoprotein phenotype consuming flaxseed oil (30 g/day, 15 g ALA per day, n6:n3 ratio <1) incorporated into cooked foods including pasta sauces, salad dressings and milk shakes (Wilkinson et al. 2005). For volunteers with type 2 diabetes, dietary cooking oil replacement with 5 g of flaxseed oil per day in unheated foods such as salads and miso soups reduced plasmin α2-plasmin inhibitor (PPI) complex level, PAI-1 activity and thrombin anti-thrombin III complex levels as compared to pretreatment levels (Tohgi et al. 2004).

Haemostatic factors (D-dimer, fibrinogen, Factor VIIa and PAI-1 activity) were not affected for healthy volunteers consuming flaxseed oil (30 ml/day, 15.9 ml ALA, n6:n3 ratio 0.25) added to foods such as porridge, yoghurt and salad dressings (Schwab et al. 2006). The fibrinolytic system was not affected for volunteers fed flaxseed oil (30 ml oil/day) (Blix et al.1965). Dietary supplements of flaxseed oil (9.2 g ALA per serving, n6:n3 ratio 0.48) emulsified in low fat milk did not affect a complete hematological screen, plama electrolytes, renal and liver function tests for normotensive, mildly hypercholesterolemic volunteers (Kestin 1990).

Animal studies

Stock diets supplemented with flaxseed or fish oil reduced collagen induced platelet aggregation to a similar extent as compared to dietary corn or coconut oil supplementation for rabbits and obese rats (Bolton-Smith et al. 1984). However, unlike fish oil supplementation, stock diets supplemented with flaxseed oil did not reduce ADP-induced aggregation as compared to dietary corn or cococut oil supplementation for rabbits and obese rats (Bolton-Smith et al. 1984).

Similar to fish oil supplementation, flaxseed oil supplemented spray dried milk powder (20.3 % ALA, n6:n3 ratio 0.33) reduced collagen and ADP-induced platelet aggregation as compared to ground nut oil supplementation for rats (Ramaprasad et al. 2005). For rabbits, a stock diet supplemented with either fish or flaxseed oil (60 g flaxseed oil/kg, 33.43 % ALA, n6:n3 ratio 0.53) reduced collagen and thrombin induced platelet aggregation as compared to corn or coconut oil supplementation. In the same study, flaxseed oil supplementation (60 g flaxseed oil/kg, 33.43 % ALA, n6:n3 ratio 0.53) reduced ADP-induced platelet aggregation as compared to corn oil supplementation and increased ADP-induced platelet aggregation as compared to fish oil supplementation (Vas Dias et al. 1982).

Coagulation factors V and VII were normalized, antifibrinolytic activity was increased and thombosis incidence was decreased for rats consuming a basal diet supplemented with saturated fat and flaxseed oil (80 mg oil/day, 47.2 % ALA, n6:n3 ratio 0.38) (Nordöy 1965). Coagulation was not affected for rats consuming a basal diet supplemented with flaxseed oil (80 mg oil/day, 47.2 % ALA, n6:n3 ratio 0.38) without the addition of saturated fat (Nordöy 1965). Hypercoagulability and hyperlipemia were

not prevented, however, thrombosis incidence and platelet adhesiveness were normalized for rats consuming a diet supplemented with saturated fat, cholesterol and flaxseed oil (80 mg oil/day) (Nordöy 1965b).

Overall, flaxseed oil supplementation up to 30 ml or 30 g of flaxseed oil/day or approximately 15 ml or 15 g of ALA per day (which far exceeds the FDA recommended adequate daily intake for ALA of 1.1 – 1.6 g/day) does not adversely affect total bleeding time or the components of the cascade of events leading to normal blood coagulation in humans. HiOmega™ flaxseed oil contains 70 % ALA, therefore, an equivalent amount of HiOmega™ flaxseed oil for 15 ml or 15 g of ALA per day would be 21 ml or 21 g of HiOmega™ flaxseed oil/day. Since such levels of flaxseed oil and ALA do not affect bleeding time, Polar Foods, Inc. has determined that, with respect to bleeding time, HiOmega™ flaxseed oil is safe within the intended uses as set forth in Table 1.

4.1.6 Glycemic Control

Markers for diabetes include hyperglycemia (fasting plasma glucose levels > 7.8 mM/L), glucosuria, polyurea, polydipsia, polyphagia, impaired glucose tolerance (plasma glucose > 11.1 mM/L with the oral glucose tolerance test), insulin resistance, hyperlipidemia, diabetic coma (Prasad 2003) and an increased risk for atherosclerotic disease (Julius 2003). Dietary supplementation with flaxseed oil does not adversely affect markers for the diabetic condition (McManus et al. 1996, Singer et al. 1986, Singer et al. 1990, Nestel et al. 1997, Nelson et al. 2007, Owren et al. 1965, Owren et al. 1964)..

Glycemic control, triglyceride, cholesterol, HDL and LDL values, insulin sensitivity, glucose effectiveness and insulin secretion were not affected for volunteers

with well-controlled, non-insulin dependent diabetes mellitus (NIDDM) consuming purified, encapsulated flaxseed oil (35 mg oil /kg body weight/day, approximately 2.85 g flaxseed oil/day, ALA 57.5% n6:n3 ratio 0.25) (McManus et al. 1996). For overweight volunteers with markers of insulin resistance on a diet supplemented with flaxseed oil based margarine and biscuits and muffins baked from the margarine (~ 54.5 g oil per day, 36.7 % ALA, 20 g ALA per day, n6:n3 ratio 0.27), the glucose area under curve (AUC) values, plasma triglyceride, LDL and total cholesterol levels were not affected, plasma insulin values levels were increased, and HDL cholesterol levels were decreased (Nestel et al. 1997). The lag time (the period before oxidation could be detected) was decreased indicating an increase in LDL oxidizability (Nestel et al. 1997). For these volunteers a functional improvement in systemic arterial circulation occurred due to increased systemic arterial compliance and decreased mean arterial pressure (Nestel et al. 1997). Fasting levels of insulin, glucose and insulin sensitivity were not affected for obese but otherwise healthy volunteers consuming a diet supplemented with flaxseed oil capsules (ALA 5 % of energy intake, 57 % ALA, n6:n3 ratio 0.32) (Nelson et al. 2007). Free fatty acid levels during the oral glucose tolerance test were not affected for hyperlipemic volunteers consuming dietary flaxseed oil (60 ml oil/day, 64 % ALA, n6:n3 ratio 0.23) during or immediately after meals (Singer et al. 1986). As compared to pre-treatment and controls, free fatty acids decreased during glucose tolerance tests for patients with type IIa, IIb, IV and V primary hyperlipoproteinemia consuming flaxseed oil (60 ml/day, 38 g ALA per day, n-6:n-3 ratio 0.23) during meals (Singer et al. 1990).

Studies which examine the effects of flaxseed oil on markers of diabetes in healthy humans and animals do not indicate adverse effects (Schwab et al. 2006,

Wilkinson et al. 2000, Carlson et al. 1975). Fasting plasma glucose levels and insulin levels were not affected for healthy volunteers consuming flaxseed oil (30 ml/day, 15.9 ml ALA, n6:n3 ratio 0.25) added to foods such as porridge, yoghurt and salad dressings (Schwab et al. 2006). Body weight, cholesterol, glucose, HDL and triacylglycerol were not affected and LDL oxidizability was reduced for normolipidemic male subjects consuming a diet supplemented with flaxseed oil (~ 30 ml oil/day, 20 g ALA/day, n6:n3 ratio of diet $\leq$ 1) (Wilkinson et al. 2000). Lower adipose tissue ALA levels were associated with increased glucose intolerance and plasma triglyceride levels for healthy male volunteers (Carlson et al. 1975).

Flaxseed oil supplementation does not adversely affect markers for diabetes in animal studies (Kleeman et al. 1998, Kabir et al. 1996, Crespo et al. 2003). For diabetes prone rats consuming a standard diet supplemented with flaxseed oil (10 % oil in diet, 41 % ALA, n6:n3 ratio 0.46), the gut associated immune system, spleen fatty acid profile, insulitis scores and IFN-γ levels were not affected and IL-10 mRNA levels did not decrease at onset of insulitis as compared to controls (Kleeman et al. 1998). Serum cholesterol levels were decreased as compared to safflower or palm oil supplementation and triacylglycerol, free fatty acid and glucose levels were decreased as compared to palm oil supplementation for rats consuming a diet supplemented with flaxseed oil (56.4 % ALA, n6:n3 ratio 0.27) (Kabir et al. 1996). Glucose concentrations were not affected whereas serum insulin, cholesterol and plasma VLDL levels were reduced as compared to olive oil or tallow supplementation for chickens consuming a diet supplemented with flaxseed oil (10 % oil, 53.8 % ALA, n6:n3 ratio 0.44) as compared to diets containing tallow, olive oil or the basal diet (Crespo et al. 2003).

Based on the published scientific literature, flaxseed oil does not adversely affect glycemic control, blood glucose, glucose tolerance or insulitis in diabetic, hyperglycemic or obese humans and does not adversely affect markers for diabetes in animal studies. Therefore, Polar Foods, Inc. has determined that HiOmega™ flaxseed oil is safe with respect to glycemic control for diabetic, hyperglycemic and healthy individuals within the intended uses as set forth in Table 1.

4.1.7 Cholesterol

Since an increased LDL or apolipoprotein B level (apo-B) is a risk factor for coronary heart disease, an increase in these levels was one of the safety concerns raised by the FDA for fish oil consumption by hypertriglyceridemic or hypercholesterolemic subjects (GRN # 138). For dyslipidaemic, hyperlipemic, hyperlipoproteinemia, hypertensive, overweight or chronically ill humans, LDL, HDL and total cholesterol are reduced, increased or not affected by dietary flaxseed oil supplementation (Singer et al. 1986, Singer et al. 1990b, Nestel et al.1997, Singer et al. 1990, Wilkinson et al. 2000, Paschos et al. 2005, Paschos et al. 2007. Rallidis et al. 2003, Rallidis et al. 2004, Kestin et al. 1990, Abbey et al.1990, Wilkinson et al. 2005, Harper et al. 2006). These mixed effects include a) total or LDL cholesterol reduced but HDL not affected b) total or LDL cholesterol not affected but HDL reduced c) total, LDL and HDL cholesterol reduced d) LDL and HDL cholesterol not affected e) LDL increased or reduced depending on polyunsaturated to unsaturated fat ratio in background diet and HDL cholesterol not affected (f) total cholesterol increased, LDL, HDL and IDL not affected.

For normal, healthy humans, dietary flaxseed oil supplementation does not affect levels of LDL, HDL and total cholesterol levels (Singer et al. 1986, Layne et al.1996, Schwab et al. 2006, Sanders et al. 1983, Mantzioris et al. 1994, Freese et al. 1997, Kelley et al. 1993, Li et al.1999, Mest et al. 1983, Pang et al. 1998). For elderly humans, dietary flaxseed oil supplementation consumed in experimental shortenings with added palm oil (21.5 % ALA, n6:n3 ratio 0.51), decreased LDL but did not affect HDL cholesterol as compared to EPA/DHA supplementation (Goyens et al. 2006).

In animals studies, dietary supplementation with flaxseed oil may reduce cholesterol, normalize or have no effect on cholesterol (Takeuchi et al. 2001, Ramaprasad et al. 2006, Ramaprasad et al. 2004, Herman et al. 1989, Yamashita et al. 2003, Jeffery et al. 1996b, Lee et al. 1988, Nordöy 1965, Vijaimohan et al. 2006, Bicknell et al. 2002, Housley et al.1986, Lee et al. 2003, Farwer et al. 1994, Landes et al. 1975, Dubey et al. 1979, Nityanand 1969, Wilson et al. 1966).

Details of specific studies for each of these cases follow.

Human studies: dyslipidaemic, hyperlipemic, hyperlipoproteinemia, hypertensive, overweight or chronically ill humans

a) total or LDL cholesterol reduced but HDL not affected

Serum total and LDL cholesterol levels were reduced whereas serum HDL cholesterol levels were not affected for hypertensive or hyperlipemic volunteers consuming a diet supplemented with flaxseed oil (60 ml oil/day, 64 % ALA, n6:n3 ratio 0.23) (Singer et al. 1986, Singer et al. 1990b). Serum total cholesterol was decreased for patients with types IIb, IV or V primary hyperlipoproteinemia consuming flaxseed oil (60 ml/day, 38 g ALA per day, n6:n3 ratio 0.23) during meals (Singer et al. 1990). LDL

oxidizability, which may contribute to atherosclerosis, was reduced and HDL cholesterol levels were not affected and for normolipidemic male subjects consuming a diet supplemented with flaxseed oil (30 ml oil/day, 20 g ALA/day, n6:n3 ratio of diet ≤ 1) (Wilkinson et al. 2000).

b) total or LDL cholesterol not affected but HDL reduced

Plasma total cholesterol and LDL cholesterol were not affected whereas plasma HDL cholesterol level was reduced for overweight volunteers with markers of insulin resistance consuming dietary supplementation with flaxseed oil and flaxseed oil margarine (~ 54.5 g oil per day, 36.7 % ALA, 20 g ALA per day, n6:n3 ratio 0.27) (Nestel et al.1997). Serum total cholesterol, LDL cholesterol, apo B and LDL density were not affected whereas serum HDL and apo A-I levels were reduced as compared to pretreatment levels for dyslipidaemic patients with certain apo E genotypes consuming flaxseed oil (15 ml/day, 8.1 g ALA/day, n6:n3 ratio 1.3) with meals (Paschos et al. 2005). Plasma total cholesterol level and LDL cholesterol were not affected whereas plasma HDL cholesterol levels were reduced for dyslipidaemic patients consuming flaxseed oil (15 ml/day, 8.1 g ALA/day, n6:n3 ratio 1.3) with meals (Rallidis et al. 2003, Rallidis et al. 2004). Plasma adiponectin and serum HDL-cholesterol levels were decreased within the flaxseed oil intervention group (15 ml flaxseed oil per day, 54.2 % ALA, n6:n3 ratio 0.26) but did not differ from the safflower oil supplemented group for dyslipidaemic male volunteers (Paschos et al. 2007b).

c) total, LDL and HDL cholesterol reduced

Plasma total cholesterol, HDL and LDL cholesterol were slightly reduced for normotensive, mildly hypercholesterolemic volunteers consuming flaxseed oil emulsified

in low fat milk (9.2 g ALA per serving, n6:n3 ratio 0.48) (Kestin et al. 1990). HDL and LDL cholesterol, Apo A-I and Apo A-II levels were decreased whereas plasma triglyceride, VLDL cholesterol, VLDL triglyceride levels, LDL and HDL particle size, thromboxane production and lipid transfer protein activity were not affected for mildly hypercholesterolemic men consuming dietary supplementation with flaxseed oil (9 g ALA/day) in a beverage (Abbey et al.1990). Plasma total cholesterol, LDL and HDL cholesterol levels were reduced for volunteers expressing an atherogenic lipoprotein phenotype consuming dietary supplements of flaxseed oil (30 g/day, 15 g ALA per day, n6:n3 ratio <1) incorporated into cooked foods including pasta sauces, salad dressings and milk shakes (Wilkinson et al. 2005).

d) LDL and HDL cholesterol not affected

LDL and HDL cholesterol levels were not affected for patients with type IIa or type IIb primary hyperlipoproteinemia consuming flaxseed oil (60 ml/day, 38 g ALA per day, n6:n3 ratio 0.23) during meals (Singer et al. 1990).

e) LDL increased or reduced depending on polyunsaturated to unsaturated fat ratio in background diet and HDL cholesterol not affected

The effect of encapsulated flaxseed oil supplementation (35 mg ALA/kg body weight/day, 57.5 % ALA) on plasma LDL cholesterol levels varied with the ratio of polyunsaturated to saturated fat in the background diet of patients with NIDDM (Goh et al. 1997). With flaxseed oil supplementation, plasma LDL cholesterol levels were increased when the diet consisted of a low polyunsaturated to saturated fat ratio but decreased when the background diet consisted of a high polyunsaturated to saturated fat

ratio (Goh et al. 1997). For these NIDDM patients, flaxseed oil supplementation did not affect plasma HDL cholesterol levels or plasma triacylglycerol levels when the background diet consisted of either a high or a low polyunsaturated to saturated fat ratio (Goh et al. 1997).

(f) total cholesterol increased, LDL, HDL and IDL not affected

Plasma LDL, HDL and IDL cholesterol levels and particle size were not affected whereas total cholesterol and larger LDL particle levels tended to increase for volunteers with multiple chronic diseases consuming encapsulated flaxseed oil (5.2 g oil/day, 58 % ALA, n6:n3 ratio: 0.29) (Harper et al. 2006).

Human Studies: Healthy Humans

Overall, dietary flaxseed oil supplementation does not affect levels of LDL, HDL and total cholesterol levels for normal, healthy humans (Singer et al. 1986, Layne et al.1996, Schwab et al. 2006, Sanders et al. 1983, Mantzioris et al. 1994, Freese et al. 1997, Kelley et al. 1993, Li et al.1999, Mest et al. 1983, Pang et al. 1998). Serum total cholesterol, LDL and HDL cholesterol levels were not affected for normal volunteers consuming flaxseed oil (60 ml oil/day, 64 % ALA, n6:n3 ratio 0.23) during or immediately after meals (Singer et al. 1986). Plasma total, LDL and HDL cholesterol were not affected for healthy volunteers consuming encapsulated flaxseed oil (oil: 35 mg/kg body weight per day, 57.5 % ALA, n6:n3 ratio: 0.25, for a 70 kg subject this would be 4.3 g oil / day, 2.47 g ALA/day) (Layne et al. 1996). Serum total cholesterol, HDL cholesterol and LDL cholesterol levels were not affected for healthy volunteers consuming flaxseed oil (30 ml/day, 15.9 ml ALA, n6:n3 ratio 0.25) added to foods such

as porridge, yoghurt and salad dressings (Schwab et al. 2006). Plasma total cholesterol and HDL cholesterol levels were not affected for healthy volunteers consuming flaxseed oil (9.38 g ALA/day, n6:n3 ratio 0.33) with meals (Sanders et al. 1983). Plasma total, HDL and LDL cholesterol were not affected for normolipidemic volunteers consuming flaxseed oil (55.6 % ALA, n6:n3 ratio 0.33) and flaxseed oil based spreads as a replacement for cooking oils and spreads (Mantzioris et al. 1994). Serum total cholesterol and HDL cholesterol levels were not affected for healthy volunteers consuming dietary encapsulated flaxseed oil supplements (10.7 g oil/day, 5.9 g/day ALA, n6:n3 ratio 0.27 on average) (Freese et al. 1997). Cholesterol levels were not affected in fasting or post prandial blood samples for healthy volunteers consuming encapsulated flaxseed oil supplements (11.9 g oil/day, 6.21 g/day ALA, n6:n3 ratio 0.27 on average) (Freese et al. 1997b). Serum total cholesterol, HDL and LDL cholesterol were not affected for healthy volunteers consuming flaxseed oil (31.7 g/day, 21.28 % ALA, ~ 6.7 g ALA/day, n6:n3 ratio 0.72) cold mixed with yogurts, salads, sandwich spreads and vegetables (Kelley et al. 1993). Plasma total cholesterol, LDL cholesterol and HDL cholesterol were not affected for healthy, vegetarian volunteers consuming flaxseed oil (15.4 g ALA per day, n6:n3 ratio 1.0) and flaxseed oil based margarines as replacement for other dietary oils (Li et al.1999). Total cholesterol, HDL cholesterol and LDL cholesterol were not affected for healthy volunteers consuming flaxseed oil (30 ml/day, 18.75 ml ALA/day, n6:n3 ratio 0.2) as part of a normal diet (Mest et al. 1983). Plasma lipid total cholesterol, LDL, HDL, HDL_2 and HDL_3 cholesterols were not affected for healthy volunteers consuming a prepared diet with flaxseed oil (10.1 g ALA/day, n6:n3 ratio 1.2) added to muffins (Pang et al. 1998).

Animal Studies

Dietary supplementation with flaxseed oil tends to reduce or normalize cholesterol for animals (Takeuchi et al. 2001, Ramaprasad et al. 2006, Ramaprasad et al. 2004, Herman et al. 1989, Yamashita et al. 2003, Jeffery et al. 1996b, Lee et al. 1988, Vijaimohan et al. 2006, Morise et al. 2004, Nordöy 1965, Wilson et al. 1966). However, some animal studies show no effect on cholesterol (Bicknell et al. 2002, Farwer et al. 1994, Landes et al. 1975, Housley et al.1986, Lee et al. 2003, Dubey et al. 1979, Nityanand 1969).

(a) Reduced cholesterol

Plasma total and HDL cholesterol levels and liver total cholesterol levels were reduced for male rats consuming a diet with flaxseed oil (20 % oil, 55.5 % ALA, n6:n3 ratio 0.28) replacing corn oil (Takeuchi et al. 2001). Plasma and liver cholesterol levels were reduced for rats consuming spray dried milk supplemented with flaxseed oil (20.3 % ALA, n6:n3 ratio 0.33) (Ramaprasad et al. 2006, Ramaprasad et al. 2004). Plasma cholesterol levels were reduced for rats consuming a basal diet supplementation with flaxseed oil (12 % oil, 51.7% ALA, n6:n3 ratio 0.37) as compared to corn oil (Herman et al. 1989). Plasma cholesterol levels were decreased for rats consuming a diet supplemented with flaxseed oil (8.8 % oil, 54 % ALA, n6:n3 ratio 0.3) as compared to sesame seed or defatted flaxseed (Yamashita et al. 2003). Serum cholesterol, triacylglycerol, nonesterified fatty acid concentrations were decreased for rats consuming a standard diet supplementated with flaxseed oil (48.8 % ALA, n6:n3 ratio 0.33) as compared to diets with higher n6:n3 ratios (Jeffery et al. 1996b). Serum cholesterol level was lowered as compared to safflower or palm oil whereas liver cholesterol level was

reduced as compared to palm oil but unaffected as compared to safflower oil for rats consuming a diet supplemented with flaxseed oil (10 % oil, 58.4 % ALA, n6:n3 ratio 0.28) as the source of dietary fat (Lee et al. 1988).

Plasma total cholesterol levels, LDL, VLDL, TC:HDL and LDL:HDL ratio were reduced for male rats consuming a high fat diet and administered flaxseed oil (1 g oil/ kg body weight, 55 % ALA, n6:n3 ratio 0.31) orally (Vijaimohan et al. 2006). Plasma glucose, insulin, total cholesterol, free cholesterol, cholesteryl ester, phospholipid, triglyceride, VLDL, LDL, VLDL-C, LDL-C and HDL-C levels were decreased and bile total cholesterol level was unaffected for hamsters consuming a commercial diet supplemented with cholesterol and flaxseed oil (12.5 % oil, 47.51 % ALA, n6:n3 ratio 0.46) (Morise et al. 2004). In this study, male hamster's bile acids, liver total cholesterol, cholesteryl ester levels and cholesterol 7α hydroxylase (CYP7A1) activity were decreased and female hamster's liver free cholesterol was decreased.

Some animal studies indicated normalizing effects of flaxseed oil supplementation on cholesterol levels. For example, the addition of dietary saturated fat increased the incidence of hypercholesterolemia for rats. However, if this high saturated fat diet was supplemented with flaxseed oil (80 mg oil/day, 47.2 % ALA, n6:n3 ratio 0.38), the incidence of hypercholesterolemia was reduced as compared to control (no oil supplementation) or corn oil supplementation (Nordöy 1965). Similarly, the increased serum cholesterol level due to an atherogenic diet with varying amounts of cholesterol was reduced for rats consuming flaxseed oil (40 % oil) as compared to corn oil, cocoa butter or dairy butter (Wilson et al. 1966).

(b) No effect on cholesterol

Serum cholesterol was not affected for n-3 deficient rats gavaged with flaxseed oil (1 ml oil/week, 53 % ALA) (Bicknell et al. 2002). Serum total cholesterol and HDL cholesterol were not affected for rats consuming a diet supplemented with flaxseed oil (40 % energy, 10 % energy as ALA, n6:n3 ratio 0.3) (Farwer et al. 1994). Serum cholesterol levels were not affected for rats consuming a diet supplemented with flaxseed oil (10 % oil, 52.59 % ALA, n6:n3 ratio 0.31) (Landes et al. 1975). Total cholesterol level was not affected for rabbits consuming a diet supplemented with flaxseed oil for 18 months (32 % energy as oil, 52.3 % ALA, n6:n3 ratio 0.26) (Housley et al.1986). Serum total cholesterol, LDL and HDL cholesterol levels and ratio of total cholesterol to HDL cholesterol did not increase for rabbits consuming a diet supplemented with flaxseed oil (5 % oil, 51-55% ALA, n6:n3 ratio approximately 0.30) (Lee et al. 2003). Serum cholesterol levels were not affected for rabbits consuming a dietary supplement of flaxseed oil (1 g oil/kg body weight) (Dubey et al. 1979) or 3 ml of flaxseed oil per day (Nityanand 1969). For rabbits, flaxseed oil supplementation with additional cholesterol increased hypercholesterolemia and formation of atherosclerotic lesions in one study (1 g oil/kg body weight) (Dubey et al. 1979) but did not affect cholesterol levels, aortic and coronary atheroma in another study (3 ml oil/day) (Nityanand 1969).

In conclusion, human and animal studies indicate dietary flaxseed oil supplementation does not tend to increase LDL cholesterol levels. The effect of flaxseed oil supplementation on chronically ill humans is mixed and there is generally no effect on cholesterol levels for healthy humans. In only one study was LDL cholesterol increased

and this effect was modulated by the ratio of polyunsaturated to saturated fat in the diet. Cholesterol levels in animal studies tend to be reduced or normalized with dietary flaxseed oil supplementation. Therefore, Polar Foods, Inc. has determined that HiOmega™ flaxseed oil, which is high in polyunsaturated fat and low in saturated fat (Tables 2 & 3), is safe in regards to LDL cholesterol levels when used as intended (Table 1).

4.1.8 Triglycerides

According to the American Heart Association (AHA website), triglycerides tend to be elevated in people who are overweight, smoke, consume excessive amounts of alcohol, are physically inactive, consume a high carbohydrate diet, have heart disease and/or diabetes. High triglyceride levels are often associated with high total cholesterol, high LDL and low HDL cholesterol levels. For humans with hyperlipoproteinemia, hypertension, hyperlipemia, hypercholesterolemia, dyslipidemia, obesity or NIDDM, dietary supplementation with flaxseed oil either reduces or has no effect on plasma or serum triglyceride levels (Singer et al.1986, Singer et al. 1990, Kestin et al. 1990, Paschos et al. 2005, Rallidis et al. 2003, Rallidis et al. 2004, Nestel et al. 1997, Goh et al.1997, Harper et al. 2006). For normal healthy humans, dietary supplementation with flaxseed oil either reduces or has no effect on plasma or serum triglyceride levels (Schwab et al. 2006, Singer et al.1986, Singer et al. 1990b, Freese et al. 1997, Kelley et al.1993, Mest et al. 1983, Sanders et al.1983, Layne et al. 1996, Mantzioris et al.1994, Wilkinson et al. 2005, Wilkinson et al. 2000). In animal studies, dietary supplementation with flaxseed oil either reduces or does not affect triglyceride levels (Herman et al. 1989,

Takeuchi et al. 2001, Farwer et al. 1994, Lee et al.1988, Dubey et al. 1979, Lee et al. 2003). Details of specific studies follow.

Human studies: hyperlipoproteinemic, hypertensive, hyperlipemic, hypercholesterolemic, dyslipidemic, obese or NIDDM humans

Serum triglycerides were reduced for hypertensive and hyperlipemic volunteers consuming flaxseed oil (60 ml oil/day, 64 % ALA, n6:n3 ratio 0.23) with meals (Singer et al.1986). Serum triglyceride levels were reduced for patients with types IIa, IIb, IV or V primary hyperlipoproteinemia consuming flaxseed oil (60 ml/day, 38 g ALA per day, n6:n3 ratio 0.23) during meals (Singer et al. 1990). Plasma triacylglyceride levels were not affected for normotensive, mildly hypercholesterolemic volunteers consuming flaxseed oil (9.2 g ALA per serving, n6:n3 ratio 0.48) emulsified in low fat milk (Kestin et al. 1990). Serum triglyceride levels were not affected for dyslipidaemic patients with certain apo E genotypes consuming flaxseed oil (15 ml/day, 8.1 g ALA/day, n6:n3 ratio 1.3) with meals (Paschos et al. 2005). Serum triglyceride levels were not affected for dyslipidaemic patients consuming flaxseed oil (15 ml/day 8.1 g ALA/day, n6:n3 ratio 1.3) with meals (Rallidis et al. 2003, Rallidis et al. 2004). Plasma triglyceride levels were not affected for overweight volunteers consuming flaxseed oil based margarine (~ 54.5 g oil per day, 36.7 % ALA, 20 g ALA per day, n6:n3 ratio 0.27) (Nestel et al. 1997). Plasma triacylglycerol levels were not affected for patients with non-insulin-dependent diabetes mellitus consuming a diet with either a high or a low polyunsaturated to saturated fat ratio and consuming encapsulated flaxseed oil (35 mg ALA/kg body weight/day, 57.5 % ALA) (Goh et al.1997). Plasma triglyceride levels were not affected

for volunteers with multiple chronic diseases consuming encapsulated flaxseed oil (5.2 g oil/day, 58 % ALA, n6:n3 ratio 0.29) (Harper et al. 2006).

Human studies: Healthy Humans

Serum total triglyceride level decreased for healthy volunteers consuming flaxseed oil (30 ml/day, 15.9 ml ALA, n6:n3 ratio 0.25) added to foods such as porridge, yoghurt and salad dressings (Schwab et al. 2006). Serum triglycerides were reduced for normal volunteers consuming flaxseed oil (60 ml oil/day, 64 % ALA, n6:n3 ratio 0.23) during or immediately after meals (Singer et al. 1986). Plasma triglyceride levels were reduced for normal, healthy volunteers expressing an atherogenic lipoprotein phenotype consuming dietary supplements of flaxseed oil (30 g/day, 15 g ALA per day, n6:n3 ratio <1) incorporated into cooked foods including pasta sauces, salad dressings and milk shakes (Wilkinson et al. 2005). Serum triglyceride levels were reduced for male volunteers with mild hypertension consuming a diet supplemented with flaxseed oil (60 ml/day, 64 % ALA (Singer et al. 1990b).

Triacylglycerol levels were not affected for normolipidemic male subjects consuming a diet supplemented with flaxseed oil (30 ml oil/day, 20 g ALA/day n6:n3 ratio of diet $\leq$ 1) (Wilkinson et al. 2000). Serum triacylglycerol levels were not affected for healthy volunteers consuming dietary encapsulated flaxseed oil supplements (10.7 g oil/day, 5.9 g/day ALA, n6:n3 ratio 0.27 on average) (Freese et al. 1997). Dietary supplementation with encapsulated flaxseed oil did not affect triglyceride levels in fasting or postprandial blood samples for healthy volunteers (11.9 g oil/day, 6.21 g/day ALA, n6:n3 ratio 0.27 on average) (Freese et al.1997b). Serum triacylglycerol levels were not affected for healthy volunteers consuming flaxseed oil (31.7 g/day, 21.28 % ALA, ~ 6.7

g ALA/day, n6:n3 ratio 0.72) cold mixed with yogurts, salads, sandwich spreads and vegetables (Kelley et al.1993). Serum triglycerides were not affected for healthy volunteers consuming flaxseed oil (30 ml/day, 18.75 ml ALA/day, n6:n3 ratio 0.2) as part of a normal diet (Mest et al. 1983). Plasma triglyceride levels were not affected for healthy volunteers consuming flaxseed oil (9.38 g ALA/day, n6:n3 ratio 0.33) with meals (Sanders et al.1983). Plasma triacylglycerides were not affected for healthy volunteers consuming encapsulated flaxseed oil (35 mg oil/kg body weight/day, 57.5 % ALA, n6:n3 ratio: 0.25, note: for 70 kg subject this would be 4.3 g oil/day, 2.47 g ALA/day) (Layne et al. 1996). Plasma triglyceride levels were not affected for normolipidemic volunteers consuming a flaxseed oil (55.6 % ALA, n6:n3 ratio 0.33) and flaxseed oil based spreads as a replacement for cooking oils and spreads (Mantzioris et al.1994).

Animal studies

Plasma triglyceride levels were reduced as compared to corn oil for rats consuming a basal diet supplementation with flaxseed oil (12 % oil, 51.7% ALA, n6:n3 ratio 0.37) (Herman et al. 1989). Plasma triglyceride and phospholipid levels were reduced and liver phospholipid levels were unchanged for male rats consuming a basal diet with flaxseed oil (20 % oil, 55.5 % ALA, n6:n3 ratio 0.28) replacing corn oil (Takeuchi et al. 2001). Serum triacylglycerol levels were reduced for rats consuming dietary supplementation with flaxseed oil (40 % energy, 10 % energy as ALA, n6:n3 ratio 0.3) as compared to sunflower-seed oil supplementation (Farwer et al. 1994). Serum triglyceride level was reduced as compared to safflower or palm oils whereas liver triglyceride level was unaffected for rats consuming a diet supplemented with flaxseed oil (10 % oil, 58.4 % ALA, n6:n3 ratio 0.28) (Lee et al.1988). Plasma triacylglycerol and

free fatty acid levels were reduced for male rats consuming a high fat diet administered flaxseed oil (1 g oil/ kg body weight, 55 % ALA, n6:n3 ratio 0.31) orally (Vijaimohan et al. 2006).

Serum triglyceride levels were not affected for rabbits consuming a dietary supplement of flaxseed oil (1g oil/kg body weight) (Dubey et al. 1979). Serum triglyceride levels were not affected for rabbits consuming diet supplemented with flaxseed oil (5 % oil, 51-55% ALA, n6:n3 ratio approximately 0.30) (Lee et al. 2003).

Lipolysis was increased during fasting for rats consuming a diet supplemented with flaxseed oil (20 % oil, 50.1 % ALA, n6:n3 ratio 0.40) (Larking et al. 1975).

In conclusion, dietary supplementation with flaxseed oil does not adversely affect triacylglycerol levels for healthy or chronically ill humans. Flaxseed oil supplementation may be beneficial as there are some indications of lowered triglyceride levels with dietary flaxseed oil in human and animal studies. The type of fat in the background diet may modulate the effect of flaxseed oil supplementation on triglyceride levels. A low/high dietary ratio of polyunsaturated/saturated fat increased the hypotriglyceridemic effect of flaxseed oil and similarly, the hypocholesterolemic effect of flaxseed oil. Therefore, Polar Foods, Inc. has determined that the intended use of HiOmega™ flaxseed oil (Table 1) which is high in polyunsaturated fat and low in saturated fat is safe with respect to triglyceride levels.

4.1.9 Blood Pressure

Dietary supplementation with flaxseed oil either reduces or does not affect blood pressure for humans with hypertension, hypercholesterolemia, dyslipidemia or obesity (Paschos et al. 2007, Nestel et al. 1997, Singer et al. 1990b, Kestin et al.1990, Venter et al. 1988, Singer et al.1986). Dietary supplementation with flaxseed oil does not affect blood pressure for normal, healthy humans (Li et al. 1999, Mest et al.1983, Schwab et al. 2006, Singer et al. 1986, Singer et al.1990b, Wilkinson et al. 2005). Dietary flaxseed oil supplementation either reduces or does not affect blood pressure for hypertensive or normal animals and increases the effect of antihypertensive medication (Rupp et al. 1996, Brändle et al. 1997, Dierberger et al.1991, Codde et al. 1984, Hoffman et al.1983, Hoffmann et al.1986, Hoffmann et al. 1985, Singer et al. 1984, Moritz et al. 1985, Ohkubo et al. 1991, Singer et al. 1986b, Singer et al. 1990c, Croft et al. 1984, Sekine et al. 2007, Mahoney et al. 1983, Dierberger et al. 1991, Hoffmann et al. 1984). Details of specific studies follow.

Human studies: dyslipidaemic, obese, hypertensive, hypercholesterolemic, hyperlipemic humans

Dietary supplementation with flaxseed oil (15 ml/day, 8 g ALA/day n6:n3 ratio 1.3) decreased systolic, diastolic and mean arterial pressure for dyslipidaemic patients (Paschos et al. 2007). Arterial compliance was increased and mean arterial pressure was decreased for overweight volunteers consuming flaxseed oil based margarine (~ 54.5 g oil per day, 36.7 % ALA, 20 g ALA per day, n6:n3 ratio 0.27) (Nestel et al. 1997). Systolic blood pressure in response to stress was decreased, diastolic blood pressure was

unaffected for male volunteers with mild hypertension consuming a diet supplemented with flaxseed oil (60 ml/day, 64 % ALA) (Singer et al. 1990b). Systolic and diastolic blood pressures were not affected for normotensive, mildly hypercholesterolemic volunteers consuming flaxseed oil (9.2 g ALA per serving, n6:n3 ratio 0.48) emulsified in low fat milk (Kestin et al. 1990). Blood pressure was not affected for non-obese patients with mild-moderate uncomplicated essential hypertension consuming encapsulated flaxseed/sunflower oil (396 mg ALA/day, n6:n3 ratio: 1.2) (Venter et al.1988). Systolic and diastolic blood pressures were not affected for hypertensive and hyperlipemic volunteers consuming flaxseed oil (60 ml oil/day, 64 % ALA, n6:n3 ratio 0.23) with meals (Singer et al.1986).

Human studies: Healthy humans

Systolic and diastolic blood pressure were not affected for healthy, vegetarian volunteers consuming dietary supplements of flaxseed oil (15.4 g ALA per day, n6:n3 ratio 1.0) and flaxseed oil based margarines as replacement for other dietary oils (Li et al.1999). Arterial blood pressure and heart rate were not affected for healthy volunteers consuming flaxseed oil (30 ml/day, 18.75 ml ALA/day, n6:n3 ratio 0.2) as part of a normal diet (Mest et al. 1983). Systolic and diastolic blood pressures were not affected for healthy volunteers consuming flaxseed oil (30 ml/day, 15.9 ml ALA, n6:n3 ratio 0.25) added to foods such as porridge, yoghurt and salad dressings (Schwab et al. 2006). Systolic and diastolic blood pressures were not affected for healthy volunteers consuming flaxseed oil (60 ml oil/day, 64 % ALA, n6:n3 ratio 0.23) with meals (Singer et al.1986). Neither systolic nor diastolic blood pressure were affected for normal, healthy volunteers expressing an atherogenic lipoprotein phenotype consuming dietary supplements of

flaxseed oil (30 g/day, 15 g ALA per day, n6:n3 ratio <1) incorporated into cooked foods including pasta sauces, salad dressings and milk shakes (Wilkinson et al. 2005).

Animal studies

Systolic and diastolic blood pressures measured in the light phase during rest were reduced for spontaneously hypertensive rats consuming dietary supplementation with flaxseed (5 % flaxseed oil wt/wt of diet, 62% ALA, n6:n3 ratio 0.24) (Rupp et al. 1996). Systolic blood pressure was decreased for hypertensive rats consuming dietary supplementation with flaxseed oil (53.3 % ALA, n6:n3 ratio 0.39) (Brändle et al.1997). Blood pressure was decreased for hypertensive rats consuming dietary supplementation with flaxseed oil (61.7 % ALA) (Dierberger et al.1991). Systolic blood pressure was reduced for one kidney one clip rats consuming a diet supplemented with flaxseed oil (40 % of calories, 50.5 % ALA, n6:n3 ratio 0.36) as compared to standard diet (Codde et al. 1984). Blood pressure was reduced for spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (62.5 % ALA, n6:n3 ratio 0.32) (Hoffman et al. 1983). Systolic blood pressure and renomedullary $PGF2_{\alpha}$ production was reduced and heart rate was not affected for spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (62.5% ALA, n6:n3 ratio 0.21) (Hoffmann et al. 1986). Blood pressure was reduced for female spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (14 % oil, 62.5 % ALA, n6:n3 ratio 0.21) (Hoffmann et al.1985). Systolic blood pressure was reduced and blood pressure reduction increased for subsequent generations for male offspring of generations of spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (14 % oil, 14.5 J % ALA, n6:n3 ratio 0.34) (Hoffmann et al. 1986b). Blood pressure was reduced for spontaneously

hypertensive rats consuming a diet supplemented with flaxseed oil (30 J % oil, 14.5 J % ALA, n6:n3 ratio 0.34) (Singer et al. 1984). Systolic blood pressure was reduced for spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (15 % oil, 64 % ALA, n6:n3 ratio 0.23) (Moritz et al.1985). Blood pressure was reduced for spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (1, 2.5 and 5 %) (Ohkubo et al.1991). Blood pressure was decreased for spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (1 ml/day, 49.3 % ALA, n6:n3 ratio 0.33) (Sekine et al. 2007). Blood pressure tended to be lower for rats consuming dietary supplementation with flaxseed oil (40 % energy, 50 % ALA) (Croft et al. 1984).

Blood pressure was not affected for normal or spontaneously hypertensive male rats consuming a diet supplemented with flaxseed oil (150 g oil/kg, 64 % ALA, n6:n3 ratio 0.23) (Singer et al. 1986b, Singer et al. 1990c). Blood pressure was not affected for rats with clipped and non-clipped kidneys consuming diet supplemented with flaxseed oil (3 % oil, 53 % ALA, n6:n3 ratio 0.26) (Mahoney et al.1983). Blood viscosity was decreased for hypertensive rats consuming dietary supplementation with flaxseed oil (61.7 % ALA) (Dierberger et al. 1991). Hypotensive effects of antihypertensive drugs were increased for rats consuming a diet supplemented with flaxseed oil (30 J % oil, 14.5 % J ALA, n6:n3 ratio 0.34) (Hoffmann et al. 1984).

In conclusion, dietary flaxseed oil supplementation does not adversely affect blood pressure in humans or animal studies. Therefore, Polar Foods, Inc. has determined that the intended use of HiOmega™ flaxseed oil as set forth in Table 1 is safe with respect to blood pressure.

4.1.10 Eicosanoids

Both α-linolenic acid and linoleic acid are substrates for the Δ6 desaturase enzyme (Fig 8). Increased α-linolenic acid intake results in reduced arachidonic acid levels (Table 12). Eicosanoids derived from arachidonic acid include prostaglandins PGH_2, PGE_2, PGF_2, prostacyclin (PGI_2), 6-keto-$PGF_{1\alpha}$, thromboxanes TXA_2 and its metabolite TXB_2, hydroperoxy-eicosatetraenoic acid (HPETE) and leukotrienes. Therefore, ALA may be a modulator of prostaglandin synthesis through competitive inhibition of the Δ6 desaturase enzyme (Marshall et al. 1983b). Most human and animal studies of the effects of dietary flaxseed oil supplementation indicated a decrease in series 1 and 2 prostaglandins and thromboxane B_2 (Mahoney et al. 1983, Codde et al. 1984b, Singer et al.1984, Croft et al. 1984, Croft et al. 1984b, Marshall et al.1982, Hubbard et al. 1994, Chartrand et al. 2003, Magrum et al. 1983, Takemura et al. 2002, Lee et al. 1988, Codde et al.1984, Marshall et al. 1985, Morris et al. 1989, Fritsche et al. 1990, Fritsche et al. 1989, Weiler et al. 2002, Ogborn et al. 2006, Hoffmann et al. 1985, Hoffmann et al. 1986, Hoffmann et al. 1986b, Caughey et al. 1996). Some human and animal studies indicate no effect (Freese et al. 1997b, Mest et al. 1983, Mueller et al. 2005, MacDonald-Wicks et al. 2002, Ingram et al. 1995, Henry et al. 1991) or increased production of eicosanoids (Rupp et al. 1996, Ohkubo et al. 1991). The suppressive effects of flaxseed oil supplementation on series 1 and 2 prostaglandin synthesis occur within days (Marshall et al. 1983b). Details of specific studies follow.

Human studies

Thromboxane B_2 (TXB_2) and prostaglandin E_2 (PGE_2) production was decreased for healthy volunteers consuming flaxseed oil (56 % ALA, n6:n3 ratio 0.3) and flaxseed oil/butter spread (Caughey et al. 1996). Dietary supplementation with encapsulated flaxseed oil did not affect TXB_2 levels measured in fasting or postprandial blood samples from healthy volunteers (11.9 g oil/day, 6.21 g/day ALA, n6:n3 ratio 0.27 on average) (Freese et al. 1997b). Arachidonic acid induced TXB_2 formation was not affected for healthy volunteers consuming flaxseed oil (30 ml oil/day, 18.75 ml ALA/day, n6:n3 ratio 0.2) as part of a normal diet (Mest et al. 1983).

Animal studies

Renin-Angiotensin system

Prostaglandin synthesis and the renin-angiotensin system are interrelated such that angiotensin II causes the release of prostaglandins and prostaglandins may induce renin release (Bolger et al. 1976). Urinary 6 keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and PGE_2 levels were suppressed for rats consuming a diet supplemented with flaxseed oil (20 % energy, 44 % ALA, n6:n3 ratio 0.5) as compared to hydrogenated coconut oil or safflower oil (Codde et al. 1984b). In this study, over a range of angiotensin II stimulation, vascular resistance was lower for flaxseed oil supplemented rats as compared to rats supplemented with safflower oil. Also, renal vascular renin activity was suppressed in both the flaxseed oil and safflower rats as compared to the hydrogenated coconut oil supplemented rats. Furthermore, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) levels were elevated after angiotensin II stimulation for the flaxseed oil supplemented rats as

compared to either the safflower or hydrogenated coconut oil supplemement rats. Plasma TXB2 and angiotensin II levels were not affected whereas plasma 6-keto-$PGF_{1\alpha}$, bradykinin and nitric oxide metabolite levels were increased for spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (1 ml/day, 49.3 % ALA, n6:n3 ratio 0.33) (Sekine et al. 2007).

Spontaneously hypertensive rats

Renomedullary $PGF_{2\alpha}$ and aortic 6-keto-$PGF_{1\alpha}$ production was reduced for spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (30 J % oil, 14.5 J % ALA, n6:n3 ratio 0.34) (Singer et al.1984). Kidney medulla PGE and $PGF_{2\alpha}$ production and aorta $PGF_{2\alpha}$ and PGI_2-like production were reduced for female spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (14 % oil, 62.5 % ALA, n6:n3 ratio 0.21) (Hoffmann et al. 1985). Renomedullary $PGF_{2\alpha}$ production was reduced for spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (62.5% ALA, n6:n3 ratio 0.21) (Hoffmann et al. 1986). Aortic 6-keto-$PGF_{1\alpha}$ was increased for spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (1, 2.5 and 5 % oil) (Ohkubo et al. 1991). PGI_2-like activity was reduced for male offspring of spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (14 % oil , 14.5 J % ALA, n6:n3 ratio 0.34) (Hoffmann et al. 1986b).

Polycystic kidney disease

PGE_2 production was reduced for male and female offspring of polycystic kidney disease Han:SPRD-cy rats consuming a diet supplemented with flaxseed oil (7% oil, 52.3 % ALA, n6:n3 ratio 0.30) (Ogborn et al. 2006). PGE_2 levels were reduced for male

polycystic kidney diseased rats consuming a diet supplemented with flaxseed oil (5 % oil, 52.3 % ALA, n6:n3 ratio 0.30) (Weiler et al. 2002).

Renal ablated or one kidney one clip

Ratio of 6-keto-$PGF_{1\alpha}$ to TXB_2 was unchanged as compared to presurgery and urinary TXB_2 level was reduced for renal ablated rats consuming a diet supplemented with flaxseed oil (15 % oil) (Ingram et al. 1995). Aortic 6-keto-$PGF_{1\alpha}$ levels were not affected whereas kidney 6-keto-$PGF_{1\alpha}$ and PGE_2 levels and serum TXB_2 levels were reduced for one kidney one clip rats consuming a diet supplemented with flaxseed oil (40 % of calories, 50.5 % ALA, n6:n3 ratio 0.36) as compared to standard diet (Codde et al.1984).

Kidney homogenate

Clipped and non-clipped kidney homogenate synthesis of 6-keto-$PGF_{1\alpha}$ and PGE_2 were reduced for rats consuming a diet supplemented with flaxseed oil (3 % oil, 53 % ALA, n6:n3 ratio 0.26) (Mahoney et al. 1983). Serum TXB_2 levels were also reduced in this study. Kidney homogenate 6-keto-$PGF_{1\alpha}$ and PGE_2 levels were reduced for rats consuming dietary supplementation with flaxseed oil (40 % energy, 50 % ALA) (Croft et al. 1984). Whole body 6-keto-$PGF_{1\alpha}$ levels, kidney homogenate 6-keto-$PGF_{1\alpha}$ and PGE_2 levels were reduced and urinary excretion of 6-keto $PGF_{1\alpha}$ was increased for rats consuming a diet supplemented with flaxseed oil (5, 20 and 40 % energy, 50 % ALA, n6:n3 ratio 0.36) as compared to a diet supplemented with coconut oil (Croft et al. 1984b).

Splenocyte, spleen, other

Liver prostaglandin synthesis was decreased, thymus and spleen prostaglandin synthesis was not affected for rats consuming a diet supplemented with flaxseed oil (10 % oil, 61.8 % ALA, n6:n3 ratio 0.28) as compared to corn oil supplementation (Marshall et al.1982). Control and phytohemaglutinin stimulated peripheral blood mononuclear cell and splenocyte PGE_2 synthesis was reduced for rats consuming a diet supplemented with flaxseed oil (10 % oil, 61.8 % ALA, n6:n3 ratio 0.28) as compared to corn oil or hydrogenated coconut oil (Marshall et al. 1985). Splenocyte PGE synthesis was decreased for mice consuming dietary supplementation with flaxseed oil (10 % flaxseed oil, 49.7% ALA, n6:n3 ratio 0.5) (Fritsche et al. 1989).

Macrophages

Plasma TXB_2 and 6-keto-$PGF_{1\alpha}$ concentration were not affected for horses fed a dietary supplementation with flaxseed oil (8% oil) and infused with endotoxin (Henry et al. 1991). Peritoneal macrophage production of thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) induced by endotoxin were reduced for horses consuming a diet supplemented with flaxseed oil (8 % by weight, 60 % ALA, n6:n3 ratio 0.28) (Morris et al. 1989). *In vitro*, peritoneal macrophages isolated from rats consuming a diet supplemented with flaxseed oil (8% oil, 45.52 % ALA, n6:n3 ratio 0.47), decreased production of TXB_2 and 6 keto-$PGF_{1\alpha}$ in response to endotoxin (Moore et al. 1991). Macrophage PGE_1 and PGE_2 synthesis was decreased for rats consuming a diet supplemented with flaxseed oil (10 % oil, 61.8% ALA, n6:n3 ratio 0.28) (Magrum et al. 1983). Peritoneal macrophage production of PG stimulated cAMP, number of high and low affinity PGE_2 binding sites

and high and low affinity binding site Kd values were unaffected for rats consuming a diet supplemented with flaxseed oil (12.5 % oil, 47.4 % ALA, n6:n3 ratio 0.51) (Opmeer et al. 1984).

Reproduction

Maternal plasma PGE_2 and uterine fluid $PGF_{2\alpha}$ levels were decreased for pigs consuming dietary supplementation with flaxseed oil (5% oil/weight diet, 50.4 % ALA, n6:n3 ratio) (Chartrand et al. 2003).

Exposure to toxins or radiation

8-iso-$PGF_{2\alpha}$ production was not affected for rats exposed to carbon tetrachloride and consuming diet supplemented with flaxseed oil (20 % oil, 50.7 % ALA, n6:n3 ratio 0.29) (MacDonald-Wicks et al. 2002). PGE_2 levels were reduced for UVB irradiated mice consuming a diet supplemented with flaxseed oil (10% oil, 48.4 % ALA, n6:n3 ratio 0.46) (Takemura et al. 2002).

Miscellaneous

6-keto-$PGF_{1\alpha}$ levels were increased and correlated with ALA levels for rats consuming dietary supplementation with flaxseed oil (2.5 or 5 % oil wt/wt of diet, 62% ALA, n6:n3 ratio 0.24) whereas TXB_2, $PGF_{2\alpha}$ and PGE_2 levels were not affected (Rupp et al. 1996). Serum TXB_2 levels and aortic 6-keto-$PGF_{1\alpha}$ levels were reduced as compared to either safflower or palm oils for rats consuming flaxseed oil (10 % oil, 58.4 % ALA, n6:n3 ratio 0.28) as the source of dietary fat (Lee et al. 1988). PGE synthesis was decreased for mice consuming dietary supplementation with flaxseed oil (10 % of diet by weight, 47 % ALA, n6:n3 ratio 0.36) (Hubbard et al. 1994). For rats, decreased PGE_2 and $PGF_{2\alpha}$ synthesis occurred within days of dietary supplementation with flaxseed

oil (10 % oil, 61.8 % ALA, n6:n3 ratio 0.28) and this change was reversible with corn oil supplementation (Marshall et al. 1983b).

Leukotrienes

Leukotriene production was increased (Cleland et al. 1990, Hubbard et al.1994), decreased (Hubbard et al.1994, Fritsche et al. 1989) or not affected (Fritsche et al. 1992, Mueller et al. 2005) with flaxseed oil supplementation.

LTB5 production was increased in peritoneal exudates cells from rats consuming dietary supplementation with flaxseed oil (10 % oil w/w diet, 47 % ALA, n6:n3 ratio 0.36) (Cleland et al. 1990). Thioglycollate-elicited murine macrophage production of LTC4 was decreased whereas production of LTC5 was increased for mice consuming dietary supplementation with flaxseed oil (10 % of diet by weight, 47 % ALA, n6:n3 ratio 0.36) (Hubbard et al. 1994). Splenocyte LTC4 production was decreased for mice consuming dietary supplementation with flaxseed oil (10 % oil, 49.7% ALA, n6:n3 ratio 0.5) (Fritsche et al. 1989). Total LTB release was not affected for chicks consuming a diet supplementated with flaxseed oil (48.9 % ALA, n6:n3 ratio 0.52) (Fritsche et al. 1992). PGE_2 and LTB_4 production was not affected for dogs consuming a dietary supplement of flaxseed oil (200 mg oil/kg/day, 570 mg ALA, n6:n3 ratio 0.30) (Mueller et al. 2005).

In conclusion, arachidonic acid (Table 12) and eicosanoids derived from arachidonic acid tend to be reduced with flaxseed oil supplementation. Polar Foods, Inc. has determined the reduction of arachidonic acid induced eicosanoids is not a safety concern and that the intended uses of HiOmega™ flaxseed oil is safe with respect to eicosanoid production.

4.1.11 Cardiovascular Health

Factors which indicate or affect cardiovascular health which have not been dealt with in other sections of this GRAS notification are discussed here.

Thrombosis

As reviewed in Hornstra et al. (1979) flaxseed oil has an antithrombotic effect in animal studies. Thrombosis incidence was normalized for rats consuming a diet supplemented with saturated fat, cholesterol and flaxseed oil (80 mg oil/day) (Nordöy 1965b). The incidence of thrombosis was reduced for rats consuming a basal diet supplemented with flaxseed oil (80 mg oil/day, 47.2 % ALA, n6:n3 ratio 0.38) and additional saturated fat (Nordöy 1965). In this same study, the incidence of thrombosis was not affected for rats consuming a basal diet supplemented with flaxseed oil (80 mg oil/day, 47.2 % ALA, n6:n3 ratio 0.38) without the additional saturated fat (Nordöy 1965). Similarly, the incidence of thrombosis induced by ADP was reduced for rats consuming a long-term diet supplemented flaxseed oil (8 % oil, 56.6 % ALA, n6:n3 ratio 0.25) and additional saturated fat (Nordöy et al. 1968). Atherosclerotic plaque formation was prevented for rabbits consuming a diet supplemented with flaxseed oil (5 % oil, 51-55% ALA, n6:n3 ratio ~ 0.30) (Lee et al. 2003). Aortic and coronary atheromas were not affected for rabbits consuming a diet supplemented with flaxseed oil (3 ml oil/day) and additional cholesterol (Nityanand 1969).

C-reactive protein

Serum c-reactive protein levels were reduced for dyslipidaemic patients consuming flaxseed oil (15 ml oil/day, 8.1 g ALA/day, n6:n3 ratio 1.3) with meals (Rallidis et al. 2003, Paschos et al. 2005). C-reactive protein levels were not affected for

abdominally obese, sedentary but otherwise healthy volunteers consuming flaxseed oil capsules for eight weeks (~11 g ALA/day, 57 % ALA, n6:n3 ratio 0.32) (Nelson 2007b). Higher ALA intake as assessed by a dietary history questionnaire is associated with lower c-reactive protein levels for healthy female volunteers (Yoneyama et al. 2007).

Other

ALA intake through dietary sources may reduce risk of coronary heart disease particularly when intake of long chain polyunsaturated fatty acids is low (Mozaffarian et al. 2005). Coronary and aortic blood flow were not affected whereas cardiac rate (beats per minute) and oxidation of labeled palmitate was decreased and ejection rate (ml/beat) and incorporation of palmitate into triglycerides was increased for male rats consuming a diet supplemented with flaxseed oil (10 % oil, 53.5 % ALA, n6:n3 ratio 0.33) (Demaison et al. 1991). Heart plasmalogen (specifically phosphoethanolamine and phosphatidylenthanolamine) levels were increased for rats consuming a diet supplemented with flaxseed oil (56.71 % ALA, n6:n3 ratio 0.39) (Barceló-Coblijn et al. 2005). Aorta, heart, liver and kidney histology were not affected for rabbits consuming a diet supplemented with flaxseed oil (3 ml oil/day) (Nityanand 1969).

Arrythmias

Ventricular premature contractions, a possible marker for arrhythmogenic right ventricular cardiomyopathy, were reduced > 85 % for half of the dogs receiving encapsulated flaxseed oil supplements (2 g oil/day, 56.2 % ALA, n6:n3 ratio 0.28) although total reduction in VPC for all dogs did not reach significance (Smith et al. 2007). Similar to EPA or DHA effects, intravenous infusion of purified ALA prevented

fatal ventricular arrhythmias in exercising dogs (Billman et al. 1999). A patent was filed based on the anti-arrhythmic effects ALA (Leaf et al. 1996).

Overall, no adverse effects on cardiovascular health were noted with flaxseed oil dietary supplementation. Polar Foods, Inc. has determined that HiOmega™ flaxseed oil is safe with respect to cardiovascular health.

4.1.12 Immune System

Immunosuppressive effects were a concern raised by the FDA regarding dietary fish oil (EPA + DHA) supplementation (GRN # 138) Human studies of the effects of dietary flaxseed oil supplementation or ALA levels on the immune system indicate mixed effects including no effect (Kelley et al. 1991, Wallace et al. 2003, Thies et al. 2001, Thies et al. 2001b, Rossetti et al. 1997, Healy et al. 2000, Nordström et al.1995), immunostimulatory effects (Paschos et al. 2007b, Kew et al. 2003) or immunosuppressive effects (Caughey et al. 1996, Kelley et al.1991, Rallidis et al. 2003, Paschos et al. 2005). Dietary flaxseed oil also has mixed effects in animal studies including no effect (Fritsche et al. 1992, Kelley et al. 1988, Fritsche et al. 1991, Jeffery et al. 1996b, Korotkova et al. 2004b, Henry et al.1991, Benquet et al. 1994, Henry et al. 1990, Cohen et al. 2005, Berger et al.1993, Hubbard et al. 1994), immunostimulatory effects (Fritsche et al. 1990, Fritsche et al. 1989, Kelley et al. 1988, Moore et al. 1991, Turek et al.1991, Turek et al.1994, Jeffery et al.1996b, Hillyer et al. 2006, Berger et al.1993, Hillyer et al. 2002, Kelley et al.1988) or immunosuppressive effects (Jeffery et al. 1996b, Marshall et al. 1985, Jeffery et al.1996). Details of specific human and animal studies follow.

Human studies

(i) Peripheral blood mononuclear cells (PBMNC)

Peripheral blood mononuclear cells (PBMNC) proliferation was suppressed with T cell mitogens (phytohemagglutinin or concanavalin A) for healthy volunteers consuming flaxseed oil (31.7 g ALA/day, n6:n3 ratio 0.72) mixed with yogurts, salads, sandwich spreads and vegetables (Kelley et al. 1991). However, in the same study, PBMNC proliferation was not affected with or without B cell mitogens (protein A or pokeweed) (Kelley et al. 1991). Furthermore, these authors showed that lymphocyte, monocyte and granulocyte levels in peripheral blood and counts of circulating T cells (Leu-4 and CD3), helper inducer cells (Leu-3a, CD4), suppressor/cytotoxic cells (Leu-2a, CD8) and B cells (Leu-12, CD19) were not affected at this high level of ALA supplementation (Kelley et al. 1991). As well, this level of ALA supplementation did not affect serum IgG, IgA, IgM, complement fractions C3 and C4 and salivary IgG levels (Kelley et al. 1991).

Other studies have examined the effects of dietary flaxseed oil on peripheral blood mononuclear cells. PBMNC production of cytokines TNF-α, IL-1β and IL-6 were not affected for healthy volunteers consuming an encapsulated flaxseed oil/palm oil /sunflowerseed oil blend (2 g ALA/day) (Thies et al. 2001). As well, lymphocyte, monocyte, leukocyte, neutrophil, eosinophil and basophil count, and neutrophil and monocyte phagocytosis and superoxide production were not affected for healthy volunteers consuming an encapsulated flaxseed oil/palm/sunflowerseed oil blend (2 g ALA/day) (Thies et al. 2001). PBMNC production of TNF-α, IL-1β, IL-6 in response to

lipopolysaccharide and production of IL-2, IL-4, IFN-γ and IL-10 in response to concanavalin A was not affected for healthy volunteers consuming a diet supplemented with encapsulated flaxseed oil (3.5 g ALA/day, n6:n3 ratio 0.36 of oil, n6:n3 of total diet 3.04) (Wallace et al. 2003). Furthermore, PBMNC phospholipid fatty acid composition, total number of lymphocyte, proportion of T lymphocytes, B lymphocytes, Th lymphocytes, Tc lymphocytes and memory Th cells, IL-2 and IFN-γ production were not affected for healthy volunteers consuming a diet supplemented with flaxseed oil (2.94 ± 0.17 g ALA/day, n6:n3 ratio 0.30) (Thies et al. 2001b). However, another study found that with increasing ALA concentration in PBMNC phospholipids for healthy volunteers, immune cell functional responses such as neutrophil and monocyte phagocytosis and oxidative burst and lymphocyte cytokine production are increased (Kew et al. 2003).

(ii) Tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β)

TNF-α and IL-1β production were decreased for healthy volunteers consuming a diet supplemented with flaxseed oil (56 % ALA, n6:n3 ratio 0.3) and flaxseed oil and butter spread (Caughey et al. 1996). However this effect was not seen in other studies. For example, PBMNC production of TNF-α and IL-1β in response to lipopolysaccharide was not affected for healthy volunteers consuming a diet supplemented with encapsulated flaxseed oil (3.5 g ALA/day, n6:n3 ratio 0.36 of oil, n6:n3 of total diet 3.04) (Wallace et al. 2003). Also, inflammatory markers such as TNF-α and serum amyloid-A were not affected for abdominally obese, sedentary but otherwise healthy volunteers consuming flaxseed oil capsules for eight weeks (~11 g ALA/day, 57 % ALA, n6:n3 ratio 0.32) (Nelson 2007b). Furthermore, plasma TNF-α was increased within the intervention group for dyslipidemic male volunteers consuming a diet supplemented with flaxseed oil (15 ml

per day, 54.2 % ALA, n6:n3 ratio 0.26) but was not affected by flaxseed oil supplementation when compared with the control group (Paschos et al. 2007b).

(iii) Interleukin-6 (IL-6) and macrophage colony stimulating factor

Serum IL-6 (Rallidis et al. 2003) and macrophage colony stimulating factor levels (Paschos et al. 2005) were reduced for dyslipidemic patients consuming flaxseed oil (15 ml/day, 8.1 g ALA/day, n6:n3 ratio 1.3) with meals. However, a slightly higher level of ALA supplementation did not affect levels of IL-6 for abdominally obese, sedentary but otherwise healthy volunteers consuming flaxseed oil capsules for eight weeks (~11 g ALA/day, 57 % ALA, n6:n3 ratio 0.32) (Nelson 2007b). Also, PBMNC production of IL-6 in response to lipopolysaccharide was not affected for healthy volunteers consuming a diet supplemented with encapsulated flaxseed oil (3.5 g ALA/day, n6:n3 ratio 0.36 of oil, n6:n3 of total diet 3.04) (Wallace et al. 2003) and PBMNC production of IL-6 was not affected for healthy volunteers consuming an encapsulated flaxseed oil/palm/sunflowerseed oil blend (2 g ALA/day) (Thies et al. 2001).

Further human studies which show no effect

T lymphocytes (helper or cytotoxic), B lymphocytes and natural killer cell levels were not affected for healthy volunteers consuming a diet supplemented with encapsulated flaxseed oil (3.5 g ALA/day, n6:n3 ratio 0.36 of oil, n6:n3 of total diet 3.04) (Wallace et al. 2003). Total number of natural killer cells, leukocytes, lymphocytes, T lymphocytes and activity of natural killer cells were not affected for healthy volunteers consuming an encapsulated flaxseed oil/palm oil/sunflower oil blend (4 g oil/day, 2 g ALA/day) (Thies et al. 2001c). Lymphocyte proliferation was not affected for healthy volunteers consuming single dose supplement of flaxseed oil (10 g oil, 57 % ALA, n6:n3

ratio 0.28) (Rossetti et al. 1997). Neutrophil chemotaxis and superoxide radical generation (intial rate and total production) were not affected for healthy volunteers consuming a diet supplemented with encapsulated flaxseed oil (9 g/day, 4.0 g ALA/day, n6:n3 ratio 0.37) (Healy et al. 2000). Clinical parameters, disease condition and pain tenderness score did not improve for patients with rheumatoid arthritis consuming a diet supplemented with flax oil powder (30 g oil powder, 32 % ALA) (Nordström et al.1995).

Animal Studies: immunosuppressive effect

Antibody dependent cell cytotoxicity by splenocytes was decreased for chicks consuming a diet supplemented with flaxseed oil (48.9 % ALA, n6:n3 ratio 0.52) (Fritsche et al. 1992). Spleen lymphocyte proliferation stimulated by conconavalin A, natural killer cell activity and popliteal lymph node weight were decreased for rats consuming a standard diet supplementated with flaxseed oil (48.8 % ALA, n6:n3 ratio 0.33) as compared to sunflower oil (Jeffery et al. 1996b). Control and phytohemaglutinin stimulated PBMNC levels decreased for rats consuming a diet supplemented with flaxseed oil (10 % oil, 61.8 % ALA, n6:n3 ratio 0.28) (Marshall et al. 1985). Endotoxin-induced cell associated TNF activity was decreased for rats consuming a diet supplemented with flaxseed oil (10 % oil, 45.8 % ALA, n6:n3 ratio 0.43) (Carrick et al. 1994). Among other effects, delayed type hypersensitivity response against human serum albuin was decreased for pups of rats consuming a dietary supplemented with flaxseed oil and exposed to antigen (7 % oil, 33 % ALA, n6:n3 ratio 0.42) (Korotkova 2004b). Rate of spleen lymphocyte proliferation was decreased and graft vs host response tended to decrease for rats consuming dietary supplementation with flaxseed oil (20 % oil, n6:n3 ratio 0.3) (Jeffery et al. 1996). Phagocytosis was suppressed for exercised

female mice consuming a diet supplemented with flaxseed oil (10% oil, 53.3 % ALA, n6:n3 ratio 0.35) (Benquet et al.1994).

Animal Studies: no effect

Serum cytokines (IL-1 β, IL-6 and TNF- α) were not affected for mice consuming a diet supplemented with flaxseed oil (10 % oil, 56 % ALA, n6:n3 ratio 0.27) (Cohen et al. 2005). Natural killer cell activity, lymphokine activated killer cell activity, splenic T-cell mitogen response to concanavalin A and IL-2 production were not affected for mice consuming dietary supplementation with flaxseed oil for two generations (n6:n3 ratio 0.3) (Berger et al. 1993). Immune organ weight and primary and secondary humoral immune responses were not affected for chicks consuming a diet supplemented with flaxseed oil (48.9 % ALA, n6:n3 ratio 0.52) (Fritsche et al. 1992). Spleen weight and splenocyte number were not affected for rabbits consuming a diet supplemented with flaxseed oil (48.36 % ALA, n6:n3 ratio 0.29) (Kelley et al. 1988). Proportion of CD4+, CD8+, B cells or macrophages were not affected for rats consuming a standard diet supplementated with flaxseed oil (48.8 % ALA, n6:n3 ratio 0.33) as compared to other diets with higher n6:n3 ratios (Jeffery et al. 1996b). Immune organ (spleen, thymus and bursa) weight was not affect for chicks consuming dietary supplementation with flaxseed oil (37.8 % ALA, n6:n3 ratio 0.75) (Fritsche et al. 1991). Basal and endotoxin-induced peritoneal macrophage secretion of TNF was not affected for rats consuming a diet supplemented with flaxseed oil (10 % oil, 45.8 % ALA, n6:n3 ratio 0.43) (Carrick et al. 1994). A23187-induced cell-associated TNF activity was not affected for rats consuming a diet supplemented with flaxseed oil (10 % oil, 45.8 % ALA, n6:n3 ratio 0.43) (Carrick et al. 1994). Number of T lymphocytes, total number of dendritic cells and number of CD86+

cells in the mammary gland tissues were not affected for lactating rats consuming a dietary supplemented with flaxseed oil (7 % oil, 33 % ALA, n6:n3 ratio 0.42) (Korotkova et al. 2004b). Nitric oxide, TNF-α and cytolitic capacity were not affected for mice consuming dietary supplementation with flaxseed oil (10 % of diet by weight, 47 % ALA, n6:n3 ratio 0.36) (Hubbard et al. 1994). Total white blood count was not affected for horses fed dietary supplementation with flaxseed oil (8% oil) and infused with endotoxin (Henry et al. 1991). Number of splenocytes, erythrocytes and lymphocytes were not affected for exercised female mice consuming a diet supplemented with flaxseed oil (10% oil, 53.3 % ALA, n6:n3 ratio 0.35) (Benquet et al.1994). No adverse effects were noted, mononuclear cell count and mean DNA concentration were not affected for horses consuming dietary supplementation with flaxseed oil (8% oil) (Henry et al. 1990). Gut associated immune system, spleen fatty acid profile and IFN-γ levels were not affected for diabetes prone rats consuming dietary supplementation with flaxseed oil (10 % oil in diet, 41 % ALA, n6:n3 ratio 0.46) (Kleeman et al. 1998).

Animal Studies: immunostimulatory or normalizing effects

Macrophage production of TNF-α was increased for rats consuming a diet supplemented with flaxseed oil (12.5 % of diet, 47 % ALA) (Turek et al. 1991). Superoxide response was increased for mice consuming dietary supplementation with flaxseed oil for two generations (n6:n3 ratio 0.3) (Berger et al. 1993). Peritoneal exudates cell and splenocyte cell mediated cytotoxicity was increased for mice consuming a diet supplemented with flaxseed oil (10 % oil/diet) (Fritsche et al. 1990). Splenocyte cell-mediated cytotoxicity activity and cell yields from spleen and peritoneum were increased for mice consuming a diet supplemented with flaxseed oil (10 % oil,

49.7% ALA, n6:n3 ratio 0.5) (Fritsche et al. 1989). Age-associated depression of primary cell-mediated immune response was reversed for mice consuming a diet supplemented with flaxseed oil (80 g oil/kg, 43.5 % ALA, n6:n3 ratio 0.32) (Hillyer et al. 2006). Antibody response and cell-mediated responsiveness was increased for mice consuming a diet supplemented with flaxseed oil (43.5 % ALA, n6:n3 ratio 0.32) (Hillyer et al. 2002). Peripheral blood lymphocyte proliferation and antibody production were increased whereas spleen weight and splenocyte number were not affected for rabbits consuming a diet supplemented with flaxseed oil (48.36 % ALA, n6:n3 ratio 0.29) (Kelley et al. 1988). TNF-α production was similar for pigs consuming a diet supplemented with flaxseed oil (10.5 % oil) as for pigs fed menhaden oil (Turek et al. 1994). TNF-α production, macrophage activation state and nitrite production was increased for pigs consuming flaxseed oil as compared with corn oil (Turek et al. 1994). Proportion of natural killer cells (CD16+ cells) was increased for rats consuming a standard diet supplementated with flaxseed oil (48.8 % ALA, n6:n3 ratio 0.33) as compared to diets with higher n6:n3 ratios (Jeffery et al. 1996b). A23187-induced macrophage secretion of TNF was increased for rats consuming a diet supplemented with flaxseed oil (10 % oil, 45.8 % ALA, n6:n3 ratio 0.43) (Carrick et al. 1994). White blood cell count was elevated for rats consuming a diet supplemented with flaxseed oil (8% oil, 45.52 % ALA, n6:n3 ratio 0.47) (Moore et al. 1991). Serum antibody levels in response to hen eggwhite lysozyme were increased and delayed type hypersensitivity response was increased for pigs consuming dietary supplementation with flaxseed oil (35 g oil/kg diet, n6:n3 ratio of diet: 2.55) (Bazinet et al. 2004). IgM plaque forming cell response was not suppressed for exercised female mice consuming a diet supplemented with flaxseed oil

(10% oil, 53.3 % ALA, n6:n3 ratio 0.35) (Benquet et al.1994). IL-10 mRNA levels did not decrease at onset of insulitis for diabetes prone rats consuming dietary supplementation with flaxseed oil (10 % oil in diet, 41 % ALA, n6:n3 ratio 0.46) (Kleeman et al. 1998).

Autoimmune Diseases

Beneficial effects of dietary flaxseed oil were noted in an animal model of systemic lupus erythematosus. For mice with induced systemic lupus erythematosus consuming a diet supplemented with flaxseed oil (51.7 % ALA) levels of anti-DNA and anti-cardiolipin antibodies, proteinuria and sedimentation rate were reduced, white blood cell count was increased and deposition of IgG in kidney renal mesangium was prevented (Reifen et al. 1998). Beneficial effects of dietary flaxseed oil were also noted in an anmal model of antiphospholipid syndrome. For mice with induced antiphospholipid syndrome consuming a diet supplemented with flaxseed oil, autoantibody levels and T-cell count were reduced and activated partial thromboplastin time, platelet count and fetal loss were normalized (Reifen et al. 2000).

Inflammatory Response

Soluble vascular cell adhesion molecule-1 (sVCAM-1) levels were significantly reduced for dyslipidemic patients consuming flaxseed oil (15 ml/day, 8.1 g ALA/day, n6:n3 ratio 1.3) with meals (Rallidis et al. 2004). sVCAM-1 and soluble e-selectin (sE-selectin) levels were decreased for healthy volunteers consuming an encapsulated flaxseed oil/palm/sunflowerseed oil blend (2 g ALA/day) (Thies et al. 2001).

Therefore, based on the published scientific literature, Polar Foods, Inc. has determined that there is no conclusive evidence of immunosuppressive effects of dietary

flaxseed oil and that the intended uses of HiOmega™ flaxseed oil is safe with respect to the human immune system.

4.1.13 Cancer

A review by Hooper et al. (2006) does not indicate any detrimental effects of plant sources of omega-3. The association of ALA and carotenoids, as assessed by a food frequency questionnaire, was not correlated with breast cancer risk for women (Nkondjock et al. 2004). Women's breast cancer risk was not associated with ALA content of red blood cell membranes (Shannon et al. 2007). As reviewed by Donaldson (2004), flaxseed intake lowers cancer risk.

Animal studies indicate a possible beneficial effect of dietary flaxseed oil supplementation on the growth and/or metastasis of some types of tumors (Rao et al. 2000, Thompson et al. 1996, Cameron et al. 1989, Jelinska et al. 2003, Fritsche et al.1990, Cognault et al. 2000, Wang et al. 2005, Chen et al. 2006, Dwivedi et al. 2005, Thuy et al. 2001). Other animal studies indicate no effect on tumor growth or metastasis or increased metastasis (Jelinska et al. 2003, Petrik et al. 2000, Coulombe et al. 1997, Yam et al. 1990). Tumor growth may be dependent on oxidant status (Cognault et al. 2000).

Mammary tumors

High levels of flaxseed oil consumption, resulting in a complete dietary n6:n3 ratio close to 1, slowed mammary tumor growth for mice consuming a diet supplemented with flaxseed oil (0.2 ml oil/mouse, 59.4 % ALA, n6:n3 ratio 0.23) (Rao et al. 2000). Established mammary tumor volume was reduced for rats consuming a diet

supplemented with flaxseed oil (Thompson et al. 1996). DMBA induced breast cancer incidence was reduced for mice consuming flaxseed oil as the only source of dietary fat (ad libitum, 47 % ALA, n6:n3 ratio 0.45) (Cameron et al. 1989). DMBA induced mammary tumor weight was decreased, however, tumor incidence was increased for rats consuming a diet supplemented with flaxseed oil (10 % oil/diet, 54.42 % ALA, n6:n3 ratio 0.30; diet: 33.67 % ALA, n6:n3 ratio 0.57) (Jelinska et al. 2003). Mammary tumor size and weight were decreased and survival time after primary tumor removal was increased whereas tumor incidence was not affected for mice injected with tumor cells and consuming a diet supplemented with flaxseed oil (10 % oil, 55.9 % ALA, n6:n3 ratio 0.32) (Fritsche et al.1990). Mammary tumor growth was inhibited with prooxidant (vitamins C and K_3) system whereas tumor growth was promoted with antioxidant vitamin E supplementation for female rats consuming a diet supplemented with flaxseed oil (15 % oil, 58.8% ALA, n6:n3 ratio 0.26) (Cognault et al. 2000). Hyperactivity and overexpression of fatty acid synthase is a marker for some types of human breast cancers (Menendez et al. 2004). Fatty acid synthase activity in SK-Br3 human breast cancer cells was decreased by purified ALA (Menendez et al.2004). Lymph node metastasis, tumor area and tumor cell proliferation were reduced and tumor malondialdehyde levels and tumor cell apoptosis were increased for female mice with tumors from injected estrogen receptor(-) human breast cancer cells and consuming a diet supplemented with flaxseed oil (3.65 % oil) (Wang et al. 2005). Lung metastasis incidence was reduced for mice with smaller primary mammary tumors consuming dietary supplementation of flaxseed oil (36.53 g oil/kg, 57 % ALA) (Chen et al. 2006).

Colon tumors

Colon tumor incidence, tumor multiplicity and tumor size were decreased for rats consuming a diet supplemented with flaxseed oil (15 % oil, 53 % ALA, n6:n3 ratio 0.28) (Dwivedi et al.2005). Colon aberrant crypt foci incidence was reduced for rats consuming a diet supplemented with flaxseed oil (7 and 14 % flaxseed oil, 57 % ALA) (Williams et al. 2007). APC min/+ mice are an animal model of intestinal tumorigenisis (Petrik et al. 2000). Colon and small intestine tumor size and number were not affected for APC min/+ mice consuming dietary supplementation with purified ALA (Petrik et al. 2000).

Liver tumors

Male C3H/He mice are an animal model of spontaneous liver tumorigenesis. Dietary supplementation with flaxseed oil (20 % oil, 63.3 % ALA, n6:n3 ratio 0.25) reduced liver tumor multiplicity in male C3H/He mice (Thuy et al. 2001). H59 is a variant of the Lewis lung carcinoma which preferentially metastasizes to the liver (Coulombe et al. 1997). Growth rate of the dorsal primary tumor was unaffected but liver metastasis incidence was increased for mice injected with H59 and consuming a diet supplemented with flaxseed oil (8 % oil) (Coulombe et al. 1997).

Prostate Cancer

Dietary flaxseed oil consumption and the risk of prostate cancer has not been studied, and the evidence for/against this relationship is based primarily on studies of tissue or dietary ALA levels.

A systematic review by MacLean et al. (2006) concludes that dietary intake of ALA is not associated with increased prostate cancer risk. However, as reviewed by Brouwer et al. (2004) and Astorg et al. (2004), dietary intake of ALA may be associated

with an increased risk of prostate cancer. Dietary sources of ALA may include red meat (Gann et al. 1994) and these foods have been linked to increased prostate cancer risk in some studies (Cross et al. 2007). A suggestion of an elevated risk of prostate cancer with red meat and processed meat consumption was noted in a large cohort study of US men and the relationship may be stronger for meat consumption and advanced prostate cancer (Cross et al. 2007). As reviewed by Gonzalez et al. (2006), dietary intake of fruits and vegetables is not significantly related to prostate cancer risk.

A reduced risk of prostate cancer was associated with dietary intake of ALA for certain ethnic groups particularly Latinos (Park et al. 2007). A reduced risk of prostate cancer was associated with dietary intake of ALA as assessed by food questionnaires in a large case controlled study (Bidoli et al. 2005). Dietary intake of ALA as assessed by food questionnaires was not associated with prostate cancer risk in a large prospective study (Koralek et al. 2006). Dietary intake and serum levels of ALA were not associated with prostate cancer risk in a case-control study of smokers (Männistö et al. 2003). Dietary intake of ALA as assessed by food questionnaires was not associated with risk of total or organ-confined prostate cancer in a large cohort study, however, ALA intake was associated with increased risk of advanced prostate cancer (Leitzmann et al. 2004). Prostate tissue levels of ALA were not correlated with locally advanced prostate carcinoma for men (Freeman et al. 2004). Prostatic ALA level was lower and not associated with serum prostate specific antigen levels for men with prostate cancer (Freeman et al. 2000). Serum levels of ALA were not associated with advanced prostate cancer risk or deaths from prostate cancer, however, ALA levels were positively associated with risk of prostate cancer (Harvei et al.1997). Higher serum levels of ALA

were associated with lower levels of serum prostate-specific antigen for Jamaican men (Ritch et al. 2007). Serum levels of ALA were not different between patients with or without prostate cancer as determined by needle biopsy (Ritch et al. 2007). Unsaturated fats were not associated with prostate cancer risk (Kolonel et al. 1988).

Plasma ALA levels were correlated with increased risk of prostate cancer in a prospective study however the authors state these results are tentative and preliminary (Gann et al. 1994). For men diagnosed with prostate cancer, erythrocyte membrane ALA levels were not different between cases and controls whereas the highest quartile of ALA and LA levels were associated with increased risk of prostate cancer (Newcomer et al. 2001). Dietary intake of ALA as assessed by food frequency questionnaires was associated with advanced prostate cancer risk in a multi-variate model (Giavannucci et al. 1993). Purified ALA stimulated growth for some but not all prostate cancer cell lines *in vitro* (Pandalai et al. 1996). Serum prostate specific antigen levels were positively correlated with prostate tissue ALA levels but not correlated with leukocyte ALA levels for men with benign prostate hyperplasia (Christensen et al. 2006). The authors comment that ALA appears to be stored in prostate tissue in the diseased state.

Arachidonic acid stimulates *in vitro* prostate cancer cell growth (Ghosh et al. 1997). It is thought that increased ALA levels are associated with decreased arachidonic acid levels (Table 12) through competitive inhibition of the linoleic acid precursor. It is possible that ALA levels would be increased to reduce the production of arachidonic acid (Faas et al. 2003).

Other effects

PGE synthesis was reduced for mice injected with tumor cells consuming a diet supplementated with flaxseed oil (10 % flaxseed oil, 55.9 % ALA, n6:n3 ratio 0.32) (Fritsche et al. 1990).

EL4-lymphoma tumor growth was inhibited as compared to soyabean or fish oil whereas tumor incidence was not affected by any dietary source of oil for male mice with transplanted tumors consuming a basal diet supplemented with flaxseed oil (4 % oil, 2.15 % ALA in diet, n6:n3 ratio 0.79 in diet) (Yam et al. 1990).

A diet/cancer interaction on blood glucose and insulin levels was noted for mice with transplanted lymphomas and thymomas (Yam et al.1990). If the basal diet was supplemented with flaxseed oil (4 % oil, 2.15 % ALA in diet, n6:n3 ratio 0.79 in diet), male EL4-lymphoma mice had reduced blood glucose levels as compared to a diet supplemented with soyabean or fish oil. In the same study, flax oil supplemented mice with thymomas had increased blood insulin levels as compared to a diet supplemented with soyabean or fish oil (Yam et al.1990).

In conclusion, based on the published scientific literature, Polar Foods, Inc. has determined that the intended uses of HiOmega flaxseed oil is safe with respect to cancer.

4.1.14 Body Weight

Flaxseed oil supplementation may decrease adipose tissue or carcass fat (Sherrington et al. 1996, Vijaimohan et al. 2006, Jeffery et al.1996b, Takeuchi et al.1995), may normalize weight gain (Veltri et al. 2006), may increase weight gain

(Nguyen et al. 2004, Chartrand et al. 2003) or, as most studies indicate, have no effect on body weight or body mass index (Hussein et al. 2005, Li et al. 1999, Wilkinson et al. 2000, Wilkinson et al. 2005, Schwab et al. 2006, Rupp et al. 1996, Landes et al. 1975, MacDonald-Wicks et al. 2002, Mahoney et al.1983, Marshall et al.1982, Rice et al. 2002, Shotton et al. 2004, Moore et al. 1991, Benquet et al. 1994, Takeuchi et al. 2001, Codde et al. 1984, Codde et al. 1984b, Landes et al. 1975, Hillyer et al. 2006, Kelley et al. 1988, Magrum et al.1983, Sankaran et al. 2007).

Mean body weight, percentage body fat and body mass index were not affected for male volunteers consuming a diet supplemented with flaxseed oil (18 g ALA/day, 56 % ALA) (Hussein et al. 2005). Dietary supplementation of flaxseed oil (15.4 g ALA per day, n6:n3 ratio 1.0) and flaxseed oil based margarines as replacement for other dietary oils did not affect body mass index, waist-to-hip ratio for vegetarian men (Li et al. 1999). Body weight was not affected for normolipidemic male subjects consuming a diet supplemented with flaxseed oil (30 ml oil/day, 20 g ALA/day n6:n3 ratio of diet ≤ 1) (Wilkinson et al. 2000). Body weight, body mass index and percentage of body fat were not affected for normal, healthy volunteers expressing an atherogenic lipoprotein phenotype consuming dietary supplements of flaxseed oil (30 g/day, 15 g ALA per day, n6:n3 ratio <1) incorporated into cooked foods including pasta sauces, salad dressings and milk shakes (Wilkinson et al. 2005). Body weight was not affected for healthy volunteers consuming flaxseed oil (30 ml/day, 15.9 ml ALA, n6:n3 ratio 0.25) added to foods such as porridge, yoghurt and salad dressings (Schwab et al. 2006). General growth characteristics such as body weight, tibia length, heart weight and ratio of heart weight-to-body weight were not affected for rats consuming dietary supplementation with

flaxseed oil (62% ALA, 1,2.5,5 % flaxseed oil wt/wt of diet, n6:n3 ratio 0.24) (Rupp et al. 1996). Food intake, body weight, organ and tissue weight were not affected for rats consuming a diet supplemented with flaxseed oil (10 % oil, 52.59 % ALA, n6:n3 ratio 0.31) (Landes et al. 1975). Average body weight and food consumption for rats exposed to carbon tetrachloride and consuming diet supplemented with flaxseed oil (20 % oil, 50.7 % ALA, n6:n3 ratio 0.29) (MacDonald-Wicks et al. 2002). Body weight gain was not affected for rats consuming diet supplemented with flaxseed oil (3 % oil, 53 % ALA, n6:n3 ratio 0.26) (Mahoney et al.1983). Body weight was not affected for rats consuming a diet supplemented with flaxseed oil (10 % oil, 61.8 % ALA, n6:n3 ratio 0.28) (Marshall et al.1982). Energy intake was not affected for rats consuming a diet supplemented with flaxseed oil (5 % or 20 % oil/diet, 59.4 % ALA, n6:n3 ratio 0.25) (Rice et al. 2002). Body and liver weight were unaffected for rats consuming a diet supplemented with flaxseed oil (11 % oil) (Shotton et al. 2004). Body weight and splanchnic vascular permeability was not affected for rats consuming a diet supplemented with flaxseed oil (8% oil, 45.52 % ALA, n6:n3 ratio 0.47) (Moore et al. 1991). Body weight was not affected for exercised female mice consuming a diet supplemented with flaxseed oil (10% oil, 53.3 % ALA, n6:n3 ratio 0.35) (Benquet et al. 1994). Body weight gain, food intake, liver weight were unaffected for male rats consuming a basal diet flaxseed oil (20 % oil, 55.5 % ALA, 0.28) replacing corn oil (Takeuchi et al. 2001). Weight gain was not affected for one kidney one clip rats consuming a diet supplemented with flaxseed oil (40 % of calories, 50.5 % ALA, n6:n3 ratio 0.36) as compared to standard diet (Codde et al. 1984). Weight gain was not affected for rats consuming a diet supplemented with flaxseed oil (20 % energy, 44 % ALA, n6:n3 ratio 0.5) as compared to hydrogenated coconut

oil/safflower oil mixture (Codde et al. 1984b). Food intake, body weight, organ and tissue weight were not affected for rats consuming a diet supplemented with flaxseed oil (10 % oil, 52.59 % ALA, n6:n3 ratio 0.31) (Landes et al. 1975). Food intake and body weight were not affected for mice consuming dietary supplementation with flaxseed oil (80g/kg, 43.5 % ALA, n6:n3 ratio 0.32) (Hillyer et al. 2006). Body weight and spleen weight were not affected for rabbits consuming dietary supplementation with flaxseed oil (48.36 % ALA, n6:n3 ratio 0.29) (Kelley et al. 1988). Body weight was not affected for rats consuming a diet supplemented with flaxseed oil (10 % oil, 61.8% ALA, n6:n3 ratio 0.28) (Magrum et al.1983). Body weight was not affected for rats with established kidney disease consuming a diet supplemented with flaxseed oil (7% oil) (Sankaran et al. 2007).

Increasing the ratio of ALA in diet limits the intra-abdominal growth of adipose depots for rats consuming a diet supplemented with flaxseed oil (amount of oil varies, n6:n3 ratio 0.3) (Sherrington et al.1996). Flaxseed oil reduced body weight and liver weight and normalized liver tissue morphology for male rats consuming a high fat diet and administered flaxseed oil (1 g oil/ kg body weight, 55 % ALA, n6:n3 ratio 0.31) orally (Vijaimohan et al. 2006). Dissectable fat, adipose deposition and popliteal lymph node weight were decreased whereas liver weight was not affected for rats consuming a standard diet supplementated with flaxseed oil (48.8 % ALA, n6:n3 ratio 0.33) as compared to sunflower oil supplemented diets or diets with higher n6:n3 ratios (Jeffery et al. 1996b). Body weight, liver weight, soleus muscle weight, carcass protein, intra-abdominal adipose tissue weight were not affected whereas carcass fat and body energy

gain was reduced for rats consuming a diet supplemented with flaxseed oil (200 g oil/kg, 54 % ALA, n6:n3 ratio 0.29) (Takeuchi et al.1995).

Weight gain was reduced for rats consuming a diet supplemented with edible tallow, high in saturated fat, and flaxseed oil as compared to diets supplemented with safflower or olive oil and flaxseed oil (Pan et al. 1993). Ketosis as measured by plasma levels of β-hydroxybutyrate was increased for rats consuming dietary supplementation with flaxseed oil as part of a ketogenic diet (55 % ALA) (Likhodii et al. 2000).

Weight gain was increased for pigs consuming a diet supplemented with flaxseed oil (5% oil, 56.9 % ALA, n6:n3 ratio 0.27) (Nguyen et al. 2004). Body fat content was increased for pigs consuming dietary supplementation with flaxseed oil (5% oil/weight diet, 50.4 % ALA, n6:n3 ratio) (Chartrand et al. 2003). Body weight gain increased and body fat normalized for lipoprotein lipase-deficient lean cats consuming a diet supplemented with flaxseed oil (30 g oil, 56.5 % ALA, n6:n3 ratio of diet 1.6) (Veltri et al. 2006).

In conclusion, Polar Foods, Inc. has determined that the intended uses of HiOmega™ flaxseed oil is safe with respect to growth and body weight.

4.1.15 Bone Health

As reviewed by Watkins et al. (2001), n-3 fatty acids which oppose the actions of eicosanoids derived from arachidonic acid may have beneficial effects on bone health in diseases such as osteoporosis, rheumatoid arthritis and osteoarthritis. A protective effect on bone metabolism was noted for volunteers consuming dietary supplementation with

flaxseed oil and walnut oil (20 g/day flaxseed oil) (Griel et al. 2007). Details of specific studies follow.

Dietary intake of a high LA:ALA ratio as assessed by food questionnaires was associated with reduced hip bone mineral density for male and female elderly volunteers (Weiss et al. 2005).

Peak load for vertebrae (LV3), femur midpoint and femur neck, bone mineral content and bone mineral density lumbar vertebrae and femurs were not affected for mice consuming a diet supplemented with flaxseed oil (10 % oil, 56 % ALA, n6:n3 ratio 0.27) (Cohen et al. 2005). Authors state that "feeding a substantial level of ALA that is attainable from a 10 % flaxseed oil diet during a period of rapid bone growth and bone modeling is safe with respect to indices of bone strength and bone mass." (Cohen et al. 2005). Femur weight, width and length, yield load, stiffness and toughness were not affected whereas femur peak load, bone mineral content and bone mineral density were increased for IL-10 knockout mice consuming a diet supplemented with flaxseed oil (10 % oil, 56 % ALA) (Cohen et al. 2005b). Flaxseed oil supplementation did not affect tibia bone strength or mineral content in mature chickens (Baird et al. 2008). Femur bone mineral content and density were increased for rats without kidney disease consuming a diet supplemented with flaxseed oil (7% oil) (Weiler et al. 2007). Bone mineral content, femur weight and length, plasma phosphate levels, parathyroid hormone and osteocalcin levels and urinary calcium and phosphate levels were unaffected whereas plasma calcium and PGE_2 levels were reduced for male polycystic kidney diseased rats consuming a diet supplemented with flaxseed oil (5 % oil, 52.3 % ALA, n6:n3 ratio 0.30) (Weiler et al. 2002). Serum IGF-1 was decreased whereas body weight, femur length, bone mineral

content, bone mineral density, bone area and bone thickness were not affected for pups of rats consuming a diet supplemented with flaxseed oil while pregnant (7 % oil, 33% ALA, n6:n3 ratio 0.42) (Korotkova et al. 2004). General growth characteristics such as body weight, tibia length, heart weight and ratio of heart weight-to-body weight were not affected for rats consuming dietary supplementation with flaxseed oil (1, 2.5, 5 % flaxseed oil wt/wt of diet, 62% ALA, n6:n3 ratio 0.24) (Rupp et al. 1996). Increasing dietary omega-3 intake results in increased omega-3 in femoral cortical bone and bone marrow for rats consuming diet supplemented with flaxseed oil (0.48 % oil, 3.12 % omega-3) (Li et al. 2003). No adverse effects on bone health were found for gilts fed a commercial diet supplemented with flaxseed oil (2.36 % ALA in diet, n6:n3 ratio of diet 1.16) (Farmer et al. 2007).

In conclusion, Polar Foods, Inc. has determined that HiOmega™ flaxseed oil is safe with respect to bone health.

4.1.16 Kidneys

Kidney disease

Pathology associated with renal injury in an animal model of kidney disease may be reduced with dietary flaxseed oil supplementation (Sankaran et al. 2007) and these effects may be modulated by gender (Ogborn et al. 2006). Serum creatinine and urea levels were not affected whereas renal cell proliferation, oxidative damage and macrophage infiltration were decreased for rats with established kidney disease consuming a diet supplemented with flaxseed oil (7% oil) (Sankaran et al. 2007). Renal histology and function was improved and cyst growth, PCNA positive cells (an indication of cell

proliferation), ox-LDL, proteinuria, creatinine clearance, glomerular hypertrophy and interstitial fibrosis were decreased for offspring of polycystic kidney disease Han:SPRD-cy rats consuming a diet supplemented with flaxseed oil (7% oil) (Sankaran et al. 2006). Cystic changes, epithelial proliferation, intestinal fibrosis, macrophage infiltration and ox-LDL detection were reduced for polycystic kidney disease Han:SPRD-cy rats consuming a diet supplemented with flaxseed oil (5% oil, 52.3 % ALA, n6:n3 ratio 0.30) (Ogborn et al. 2002). Creatinine and urine protein/creatinine ratio were not affected whereas cystic change and macrophage infiltration were reduced for male offspring of polycystic kidney disease Han:SPRD-cy rats consuming a diet supplemented with flaxseed oil (7% oil, ALA 52.3 %, n6:n3 ratio 0.30) (Ogborn et al. 2006). In the same study, female offspring of polycystic kidney disease Han:SPRD-cy rats consuming a diet supplemented with flaxseed oil (7% oil, ALA 52.3 %, n6:n3 ratio 0.30) had reduced urine protein/creatinine ratio, cyst area, fibrosis, ox-LDL, macrophages and epithelial proliferation (Ogborn et al. 2006).

Other effects

Kidney sclerosis was reduced for male rats with two kidneys consuming a diet supplemented with flaxseed oil (Gröne et al. 1989).

Glomeruli grade 2 mesangial expansion, glomerulosclerosis and urinary proteinuria remained stable for renal ablated rats consuming a diet supplemented with flaxseed oil (15 % oil) (Ingram et al. 1995).

Dietary supplements of flaxseed oil (9.2 g ALA per serving, n6:n3 ratio 0.48) emulsified in low fat milk did not affect a complete hematological screen, plasma

electrolytes or renal and liver function tests for normotensive, mildly hypercholesterolemic volunteers (Kestin et al. 1990).

Urinary sodium excretion was decreased whereas urinary potassium, creatinine, vanillmandelic acid, noradrenaline and adrenaline excretion were unaffected for male volunteers with mild hypertension consuming a diet supplemented with flaxseed oil (60 ml/day, 64 % ALA) (Singer et al. 1990b).

In conclusion, Polar Foods, Inc. has determined that the intended uses of HiOmega™ flaxseed oil is safe with respect to kidney health and function.

4.1.17 Liver

Dietary supplements of flaxseed oil (9.2 g ALA per serving, n6:n3 ratio 0.48) emulsified in low fat milk did not affect liver function tests for normotensive, mildly hypercholesterolemic volunteers (Kestin et al. 1990).

Adverse liver tissue morphological changes such as poor cellularity, indications of fatty liver and portal inflammability with large hepatocytes, occurred in male rats consuming a high fat diet. These changes were normalized for rats consuming the high fat diet and administered flaxseed oil (1 g oil/ kg body weight, 55 % ALA, n6:n3 ratio 0.31) orally (Vijaimohan et al. 2006).

Liver mitochondrial and peroxisomal fatty acid oxidation, activity of fatty acid oxidation liver enzymes and liver phospholipid were increased whereas activity of fatty acid synthetase, glucose-6-phosphate dehydrogenase and pyruvate kinase were decreased for rats consuming a diet supplemented with flaxseed oil (56.4 % ALA, n6:n3 ratio 0.27) (Kabir et al. 1996). Liver weight was unaffected and hepatic fatty acid oxidizing enzyme

activities (AST, CPT, P βOX) were increased whereas hepatic fatty acid synthesizing enzyme activities (G6PDH and ACC) and hepatic lipogenic enzyme activities (PAP, DGAT, PCDGT) were reduced for male rats consuming a basal diet with flaxseed oil (20 % oil, 55.5 % ALA, n6:n3 ratio 0.28) replacing corn oil (Takeuchi et al. 2001). For rats consuming spray dried milk supplemented with flaxseed oil (20.3 % ALA, n6:n3 ratio 0.33) liver lipid peroxide levels and catalase activity were increased as compared to ground nut oil supplementation but decreased as compared to fish oil supplementation (Ramaprasad et al. 2005). Similar to fish oil supplementation, platelet lipid peroxide levels and glutathione transferase activity were increased for rats consuming spray dried milk supplemented with flaxseed oil (20.3 % ALA, n6:n3 ratio 0.33) as compared to ground nut oil supplementation (Ramaprasad et al. 2005). With flaxseed oil supplementation (20.3 % ALA, n6:n3 ratio 0.33), rat liver glutathione peroxidase activity was decreased as compared to ground nut oil supplementation but increased as compared to fish oil supplementation (Ramaprasad et al. 2005). Liver triacylglycerol and microsomal acyl-CoA:diacylglycerol acyltransferase activity were increased whereas acyl-CoA oxidase activity and phosphatidate hydrolysis in microsomes and cytosol were unaffected for rats consuming a diet supplemented with flaxseed oil (20 % oil (99% flaxseed oil, 1 % sunflower oil))(Rustan et al. 1992). Liver microsomal HMG CoA reductase activity was reduced and bile flow was increased due to increased secretion of cholesterol, phospholipids, bile acids and uronic acid for rats consuming spray dried milk supplemented with flaxseed oil (20.3 % ALA, n6:n3 ratio 0.33) (Ramaprasad et al. 2006). Liver enzyme activities were not affected for rats force fed a zinc deficient diet supplemented with flaxseed oil (80 % oil, 56.9 % ALA, n6:n3 ratio 0.27) (Eder et al.

1994). Liver 5-hydroxytrytophan decarboxylation, monamine oxidase, catechol-O-methyltransferase and 5-hydroxytrytophan metabolism were not affected for rats consuming a diet supplemented with flaxseed oil (7% oil) (Century et al. 1968).

Liver Δ6-desaturase and Δ5-desaturase activities were increased whereas Δ9-desaturase activity tended to decrease for rats consuming a diet supplemented with flaxseed oil (20 % oil (99% flaxseed oil), 61.5% ALA, n3:n6 ratio 0.189) (Christiansen et al. 1991). Liver cholesterol, triglyceride, microsomal cholesterol and microsomal Δ6-desaturase activity level were unaffected for rats consuming flaxseed oil (10 % oil, 58.4 % ALA, n6:n3 ratio 0.28) as the source of dietary fat (Lee et al. 1988). In this same study, liver phospholipid levels were increased with flaxseed oil supplementation as compared to safflower oil supplementation but unchanged as compared to palm oil supplementation. Also, with flaxseed oil supplementation, microsomal phospholipase A2 activity was increased as compared to supplementation with either safflower or palm oils (Lee et al. 1988). Liver phosphatidylserine and cholesterol mass were reduced for rats consuming a diet supplemented with flaxseed oil (56.71 % ALA, n6:n3 ratio 0.39) (Barceló-Coblijn et al. 2005). Liver total lipid and triglyceride levels as measurements of fatty liver were not affected for rats force fed a zinc deficient diet supplemented with flaxseed oil (56.8 % ALA, n6:n3 ratio 0.26) (Eder et al. 1994).

In conclusion, Polar Foods, Inc. has determined that the intended uses of HiOmega™ flaxseed oil is safe with respect to liver health and function.

4.1.18 Reproduction

Pregnancy outcome variables were not affected for pregnant women consuming dietary supplementation with ALA enriched margarine (2.8 g ALA/day) (De Groot et al. 2004b). Length of gestation was not affected for pregnant women consuming a diet supplemented with flaxseed oil (4 g oil, 2.2 g ALA per day) (Knudsen et al. 2006). ALA is not detected in umbilical cord phospholipids but is present in maternal plasma phospholipids (Rump et al. 2001). Infant plasma phospholipid ALA levels were correlated with maternal plasma phospholipids ALA levels (Elias et al. 2001). DHA is formed from labeled purified ALA ethyl ester for human infants (Salem et al.1996). Maternal DHA status and cognitive performance were not affected for pregnant women consuming dietary supplementation of ALA in the form of margarine (2.82 g ALA/day, n6:n3 ratio 3.2) (De Groot et al. 2004).

Mammary gland structures (terminal end bud, alveolar buds and lobules), relative ovarian weight and serum estradiol were not affected for offspring and female rats consuming a diet supplemented with flaxseed oil (1.82 % oil) (Tou et al.1999). Mammary gland developmental growth was not affected for mice consuming a diet supplemented with flaxseed oil (20 % oil, 47 % ALA, n6:n3 ratio 0.51) (Welsch et al. 1989). Mammary development was not affected for gilts fed a commercial diet supplemented with flaxseed oil (2.36 % ALA in diet, n6:n3 ratio of diet 1.16) (Farmer et al. 2007). Breastmilk saturated fat levels were higher, pup body weight and length and serum leptin levels were decreased and serum glucose, protein, cholesterol, triglyceride

levels were not affected for lactating rats consuming a diet supplemented with flaxseed oil while pregnant (7% oil, 33 % ALA, n6:n3 ratio 0.42) (Korotkova et al. 2002).

Growth and reproduction were not adversely affected for rats consuming dietary supplementation with flaxseed oil (10 % oil wt/wt diet, 58.6 % ALA, n6:n3 ratio 0.3) (Brown et al. 1984). Litter size and body weight of pups at weaning were not affected and renal disease progression was reduced for offspring of polycystic kidney disease Han:SPRD-cy rats consuming a diet supplemented with flaxseed oil (7% oil) (Sankaran et al. 2006).

Body weight, femur length, bone mineral content, bone mineral density, bone area and bone thickness were not affected for pups of rats consuming a diet supplemented with flaxseed oil while pregnant (7 % oil, 33 % ALA, n6:n3 ratio 0.42) (Korotkova et al. 2004). Serum levels of protein, glucose, leptin, triacylglycerol, cholesterol, insulin and systolic blood pressure were not affected for pups of rats consuming a diet supplemented with flaxseed oil while pregnant (7% oil, 33 % ALA, n6:n3 ratio 0.42)(Korotkova et al. 2005). Fetal weight, protein and DNA content were not affected whereas protein:DNA ratio was decreased for pigs consuming dietary supplementation with flaxseed oil (5% oil/wt diet, 50.4 % ALA, n6:n3 ratio) (Chartrand et al. 2003).

Reproductive organ (ovaries and uterus) weight and appearance were not affected for female mice consuming a diet supplemented with flaxseed oil (3.65 % oil) (Wang et al. 2005). Testicular plasma total, free and esterified cholesterol and phospholipid, cholesterol to phospholipids ratio and binding capacity of the testicular plasma membrane to labeled human chorionic gonadotrophin were reduced whereas cAMP synthesis was

increased for rats consuming a diet supplemented with flaxseed oil and additional beef tallow (16 % oil, 4 % tallow, 39.9% ALA, n6:n3 ratio 0.41)(Sebokova et al. 1990).

In conclusion, Polar Foods Inc. has determined that HiOmega™ flaxseed oil is safe with respect to reproductive function.

4.1.19 Eyes

For primates, rats and guinea pigs, ALA deficient diets result in reduced brain and retina DHA levels and impairments in visual function (Abedin et al. 1999). Rod outer segment DHA levels were decreased and DPA levels increased for rats consuming a ALA deficient diet (Organisciak et al. 1986). With dietary flaxseed oil (53 % ALA, n6:n3 ratio 0.32) supplementation, DHA levels returned to the same levels as controls for n-3 deficient rats (Organisciak et al. 1996). Similarly, gavaged flaxseed oil (1 ml oil/week, 53 % ALA) reversed a drop in rod outer segment DHA levels for n-3 deficient rats (Bicknell et al. 2002). Retinal pigment epithelium and rod outer segment DHA levels were increased for rats consuming a diet supplemented with flaxseed oil (52.2% ALA, n6:n3 ratio 0.31) (Wang et al. 1992). Rod outer segment DHA levels were increased for rats reared in bright light and consuming a diet supplemented with flaxseed oil (10 % oil, 53 % ALA, n6:n3 ratio 0.38) (Wiegand et al. 1995). DHA levels were highest and DHA turnover rate was fastest for rats consuming a diet supplemented with flaxseed oil (10 % weight oil/weight diet, 53 % ALA, n6:n3 ratio 0.38) (Stinson et al. 1991). An age dependent decrease in rod outer segment DHA levels was prevented by supplementation with flaxseed oil (1 ml oil per week) for rats (Organisciak et al.1986).

Outer nuclear layer was thinner for rats consuming a diet supplemented with flaxseed oil (10 % oil wt/wt diet) (Koutz et al. 1995). Outer nuclear layer area and length of rod outer segment was not affected for female rats consuming diet supplemented with flaxseed oil (10 % oil, 53 % ALA, n6:n3 ratio 0.38) (Wiegand et al. 1991).

Greater numbers of photoreceptor cells were lost as a result of acute bright light stress for rats raised in bright light or dim light environments and consuming a diet supplemented with flaxseed oil (10 % oil wt/wt diet) (Koutz et al. 1995). Retinal light damage was reduced for rats consuming a diet deficient in ALA (Organisciak et al. 1996).

Electroretinogram responses were improved (higher a-wave responses, quicker b-wave responses) for puppies consuming either dietary supplementation with flaxseed oil or breastmilk from mothers whose diets were supplemented with flaxseed oil (68.2 % ALA) (Heinemann et al. 2005).

Plasma ALA levels were lower for patients with retinitis pigmentosa (Gong et al. 1992). P23H rats are an animal model of retinitis pigmentosa. Gavaged flaxseed oil did not affect rhodopsin or DNA levels in control or P23H rats (Bicknell et al. 2002).

Higher intake of ALA as assessed by food frequency questionnaires tended to be associated with lower incidence of age related maculopathy for older volunteers (Chua et al. 2006). However, dietary intake of linolenic acid, particularly from meat and margarine, was associated with increased risk of age related macular degeneration (Cho et al. 2001). A review of human studies by Hodge et al. (2005) stated that no conclusions could be drawn regarding ALA and human eye health.

Dietary ALA affected retinal pigment epithelial cell density and response to xanthophyll supplementation for monkeys. The authors conclude that "n-3 fatty acids appear to be essential for the development and/or maintenance of a normal density profile of retinal pigment epithelial cells in the central retina."(Leung et al. 2004).

Vitamin A

Retinol and carotenoids, collectively known as Vitamin A, are important for eye health, bone growth, reproduction and modulation of the immune system (http://ods.od.nih.gov/factsheets/vitamina.asp). Vitamin A levels in liver and serum as well as mobilization rate and retinyl ester composition in liver were not affected for rats consuming dietary supplementation of flaxseed oil (56.3 % ALA, n6:n3 ratio 0.31) (Tomassi et al. 1983). Plasma, kidney, lung, spleen and liver vitamin A levels and liver and lung β-carotene levels were not affected for female rats consuming a diet supplemented with flaxseed oil (1 ml oil) (Schweigert et al. 2000). Vitamin A levels do not affect rat liver or rod outer segment fatty acid composition (Organisciak et al.1986). Vitamin A levels in liver are affected by the quality of fat (Gronowska-Senger et al. 1978).

In conclusion, Polar Foods, Inc. has determined that the intended uses of HiOmega™ flaxseed oil is safe with respect to eye function and health.

4.1.20 Skin

Skin lesions, fatty liver and abnormal adrenal glands were healed for essential fatty acid deprived capuchin monkeys after consuming a diet supplemented with flaxseed oil (55.7 % ALA, n6:n3 ratio 0.27) (Fiennes et al.1973, Sinclair et al. 1974). Erythema

scores and the erythema plus edema scores were lower for UVB irradiated mice consuming a diet supplemented with flaxseed oil (10% oil, 48.4 % ALA, n6:n3 ratio 0.46) (Takemura et al. 2002). Clinical scores for atopic dermatitis were improved for dogs consuming a diet supplemented with flaxseed oil (200 mg/kg/day oil, 570 mg ALA, n6:n3 ratio 0.30) (Mueller et al. 2004, Mueller et al. 2005). Scaly dermatitis was reduced for an ALA deficient patient treated with ethyl linolenate (Bjerve et al. 1987).

In conclusion, Polar Foods, Inc. has determined that the intended uses of HiOmega™ flaxseed oil is safe with respect to skin health.

4.1.21 Mental Health

Hyperactivity scores were decreased for children diagnosed with attention deficit hyperactivity disorder consuming a diet supplemented with flaxseed oil (200 mg ALA/day) (Joshi et al. 2006). No serious adverse effects were noted for adults with ADHD consuming a diet supplemented with flaxseed oil (60 g oil/day, 59.6 % ALA) (Young et al. 2005).

Depression was prevented and aggression reduced for rat pups consuming dietary supplementation with flaxseed oil (1 % oil which provided 4 % ALA of total fatty acids, n6:n3 ratio of total diet: 6.0) as compared to rat pups consuming an ALA deficient diet (DeMar et al. 2006).

Fatty acids may play an important role in the management of seizures (Mostofsky et al. 2004) and ALA may have anticonvulsant effects (Dell et al. 2001). Seizure pattern was modulated and protection from pentylenetetrazole (PTZ) induced seizures was

increased for rats consuming dietary supplementation with flaxseed oil as part of a ketogenic diet (55 % ALA) (Likhodii et al. 2000).

Cranial (trigeminal) and spinal nerves (sciatic and ulnar) have different fatty acid patterns and lipid class compositions. Modification of the phospholipid fatty acid profile of spinal nerves (sciatic and ulnar) but not cranial nerves (trigeminal) followed dietary intake for rats consuming a dietary supplementation of flaxseed oil (10 % oil, 54.11 % ALA, n6:n3 ratio 0.29) (Tarozzi et al. 1991). Brain phosphatidylinositol, cardiolipin (Ptd_2Gro) and cholesterol/phospholipids ratio were reduced for rats consuming dietary supplementation with flaxseed oil (56.71 % ALA, n6:n3 ratio 0.39) (Barceló-Coblijn et al. 2005). Brain 5'-nucleotidase activity was not affected for offspring of chickens consuming a diet supplemented with flaxseed oil (34.6 % ALA, n6:n3 ratio of diet 0.85) (Anderson et al.1989).

Clinical case reports indicate dietary flaxseed oil supplementation may have beneficial effects for patients with psychiatric disorders (Rudin 1981, Rudin 1982). A review by Haag et al. (2003) notes that dietary n6:n3 ratio should be decreased for optimal mental health and that flaxseed oil supplements provide similar mental health benefits as purified EPA/DHA.

In conclusion, Polar Foods, Inc. has determined that the intended uses of HiOmega™ flaxseed oil is safe with respect to mental health.

4.1.22 Lungs

Lung 12-hydroxyeicosatetraenoic acid (12-HETE) levels were decreased for mice consuming a diet supplemented with flaxseed oil (10 % oil, 57 % ALA, n6:n3 ratio 0.28) (Zhang et al. 1996). Alveolar macrophage leucine aminopeptidase activity was increased for pigs consuming a diet supplemented with flaxseed oil (10.5 % oil, 44 % ALA, n6:n3 ratio 0.41) (Turek et al. 1996).

4.1.23 Antibiotic

A review by Das (2006) indicates that flaxseed oil (ALA) may be an effective antibiotic. Some strains of staphylococci are inhibited by linolenic acid (Lacey et al. 1981).

4.1.24 Enzyme Activity

Carnitine palmitoyltransferase, cytochrome oxidase, and catalase activities, peroxisomal β-oxidation, Na+ channel density and dissociation constant Kd, Na+, K+-ATPase activity were not affected for rats consuming a diet supplemented with flaxseed oil (200 g oil/kg, 54 % ALA) (Takeuchi et al.1996). Induced cytochrome P-450 enzymes activities were increased for male rats consuming a diet supplemented with flaxseed oil (20 % oil, 55 % ALA, n6:n3 ratio 0.32) (Chen et al.1997). Plasma selenium, superoxide dismutase and glutathione peroxidase were not affected for rats exposed to carbon tetrachloride and consuming a diet supplemented with flaxseed oil (20 % oil, 50.7 % ALA, n6:n3 ratio 0.29) (MacDonald-Wicks et al. 2002).

Radiation induced increases in lipid peroxidation, aspartate aminotransferase, alanine aminotransferease and acid phosphatase were significantly reduced, radiation induced decreases in glutathione and alkaline phophatase activities were reduced for mice receiving whole body radiation and consuming dietary supplementation with flaxseed oil (Bhatia et al. 2007). Microsomal Δ6-desaturase activity level was unaffected whereas microsomal phospholipase A_2 activity was increased for rats consuming a diet supplemented with flaxseed oil (10 % oil, 58.4 % ALA, n6:n3 ratio 0.28) (Lee et al.1988). Plasma aspartate aminotransferase activity was reduced for mice consuming a diet supplemented with flaxseed oil (20 % oil, 63.3 % ALA, n6:n3 ratio 0.25) (Thuy et al. 2001). Other effects of flaxseed oil supplementation on liver enzyme activities are noted in section 4.1.16 of this GRAS notification.

In conclusion, Polar Foods, Inc. has determined that the intended uses of HiOmega™ flaxseed oil is safe with respect to enzyme activity.

4.1.25 Blood

Red blood cell concentration was not affected for rabbits consuming a diet supplemented with flaxseed oil for 18 months (32 % energy as oil, 52.3 % ALA, n6:n3 ratio 0.26) as compared to fish, palm, olive or safflower oil supplemented diets (Housley et al. 1986). In this study, red blood cell deformability did not differ among flaxseed oil, palm, olive or safflower oil supplemented rabbits but was decreased for rabbits supplemented with fish oil. In the same study, red blood cell osmotic fragility was lower for flaxseed oil supplemented rabbits as compared to fish oil supplemented rabbits in the absence of chlorpromazine (Housley et al. 1986). Chlorpromazine tends to increase

osmotic fragility of red blood cells in humans and rabbits (Housely et al. 1986). In the presence of chlorpromazine, red blood cell osmotic fragility was not different for flaxseed oil supplemented rabbits as compared to rabbits supplemented with fish, palm, olive or safflower oils (Housely et al. 1986).

Haptoglobin levels were decreased for pigs consuming dietary supplementation with flaxseed oil (35 g oil/kg diet, n6:n3 ratio of diet: 2.55) (Bazinet et al. 2004).

4.1.26 Minerals and Vitamins

Iron utilization, plasma calcium and magnesium were unaffected whereas plasma copper was increased for rats consuming a diet supplemented with flaxseed oil (11 % oil) (Shotton et al. 2004). Calcium and phosphorus reabsorption was improved for one pig consuming a diet supplemented with flaxseed oil (Husband et al.1923).

Dietary supplementation of flaxseed oil (15.4 g ALA per day, n6:n3 ratio 1.0) and flaxseed oil based margarines as replacement for other dietary oils did not affect α-tocopherol healthy, vegetarian volunteers (Li et al. 1999). Dietary supplementation with high ALA margarine spread based on flaxseed oil (9.5 g ALA/day) had no effect on plasma α-tocopherol content or ferric-reducing antioxidant power assay for moderately hyperlipidemic but otherwise healthy volunteers (Finnegan et al. 2003). However animal studies indicate tocopherol levels are reduced with flaxseed oil supplementation. For example, serum and liver α-tocopherol were lower for rats consuming a diet supplemented with flaxseed oil (40 % energy, 10 % energy as ALA, n6:n3 ratio 0.3) (Farwer et al. 1994). Plasma vitamin E levels were lowered for rats exposed to carbon tetrachloride and consuming a diet supplemented with flaxseed oil (20 % oil, 50.7 %

ALA, n6:n3 ratio 0.29) (MacDonald-Wicks et al. 2002). Plasma and liver γ-tocopherol levels were decreased for rats consuming a diet supplemented with flaxseed oil (8.8 % oil, 54 % ALA, n6:n3 ratio 0.3) as compared to sesame seed (Yamashita et al. 2003).

4.1.27 Miscellaneous Effects

Pre and post prandial whole body oxygen consumption and norepinephrine turnover rate in interscapular brown adipose tissue were not affected for male rats consuming a diet supplemented with flaxseed oil (200 g oil/kg, 53.9 % ALA, n6:n3 ratio 0.29) (Takeuchi et al. 1995b).

Adiponectin levels decreased for obese but otherwise healthy volunteers consuming a diet supplemented with flaxseed oil capsules (ALA 5 % of energy intake, 57 % ALA, n6:n3 ratio 0.32) (Nelson et al. 2007).

4.1.28 Oxidative Stability

Sensory and oxidative quality of cold pressed flaxseed oil may depend upon the method of processing the flaxseed and the subsequent storage of the flaxseed oil (Bruhl et al. 2007, Choo et al. 2007, Wiesenborn et al. 2005). Edible oils consist mainly of triacylglycerols (95 %). Non-TAG or unsaponifiable matter makes up the remaining 5 %. These minor components are naturally occurring compounds with antioxidative properties that help protect oils against oxidative deterioration. Minor components of vegetable oils in general include phospholipids, tocols, phenolic compounds, pigments (carotenoids, chlorophylls), sterols, free fatty acids, mono-acylglycerols and di-acylglycerols (Abuzaytoun et al. 2006). Cyclolinopeptide A is a minor constituent found

specifically in flaxseed oil which has effects similar to cyclosporine A (Wieczorek et al. 1991). No toxic effects were noted from intraperitoneal, per os and intravenous application of cyclinopeptide A in mice and rats (Wieczorek et al. 1991). Removing minor constituents tends to reduce the stability of the flaxseed oil. Stored oils tend to develop a bitter taste which has been attributed to cyclopeptide E (Brühl et al. 2007). The stability of HiOmega™ flaxseed oil is similar to conventional flaxseed oil. HiOmega flaxseed oil was comparable to conventional flaxseed oil in overall odor and flavor after accelerated and practical (six months) storage testing. The odor/flavor of HiOmega flaxseed oil and conventional flaxseed oil stored for six months was described as slightly nutty with some aftertaste. Cold pressed HiOmega™ flaxseed oil and conventional flaxseed oil in stored in the refigeratated nutraceutical section of the marketplace carries a shelf life of 4 – 18 months, with 12 months being commonplace for flaxseed oil packed in inert gas containers.

Thiobarbituric acid-reactive substances (TBARs) and headspace volatiles are used for monitoring secondary products of oxidation. TBARs level may indicate the formation of malondialdehyde, a mutagenic and carcinogenic compound that is a product of lipid peroxidation and prostaglandin biosynthesis (Eritsland 2000, Marnett 1999). Malondialdehyde is used to measure the susceptibility of LDL to oxidative modification (Eritsland et al. 2000). Serum malondialdehyde levels and white blood cell chemiluminescence did not increase whereas aortic malondialdehyde levels and chemiluminescence were increased for rabbits consuming a diet supplemented with flaxseed oil (5 % oil, 51-55% ALA, n6-n3 ratio approximately 0.30) (Lee et al. 2003). The ex-vivo susceptibility of LDL to oxidation was higher for EPA+DHA supplemented

diets (1.7 g EPA+DHA/day) than for diets supplemented with high ALA margarine spread based on flaxseed oil (9.5 g ALA/day) (Finnegan et al. 2003).

Plasma TBARS concentration was not affected for healthy volunteers an encapsulated flaxseed oil/palm oil/sunflower oil blend (4 g oil/day, 2 g ALA/day) (Thies et al. 2001c). TBARS level in the presence of butylated hydroxytoluene was increased for UVB irradiated mice consuming a diet supplemented with flaxseed oil (10% oil, 48.4 % ALA, n6:n3 ratio 0.46) (Takemura et al. 2002). Composition (triacylglycerol, cholesterol ester, cholesterol, phospholipids and protein) and diameter of lymph chylomicrons were not affected however oxidized lymph chylomicron TBARs level and plasma clearance was increased for rats infused with flaxseed oil through a gastric cannula (300 mg oil, 53 % ALA, n6:n3 ratio 0.28) (Umeda et al. 1995). Plasma and liver lipid peroxide levels were increased for mice consuming a diet supplemented with flaxseed oil (20 % oil, 63.3 % ALA, n6:n3 ratio 0.25) (Thuy et al. 2001). However, the amount of oxidative stress in a diet supplemented with flaxseed oil is not different than other diet types (MacDonald-Wicks et al. 2002). In one study, LDL oxidizability was reduced as compared to the usual diet for healthy male volunteers consuming 30 ml of flaxseed oil (20 g ALA) for several weeks (Wilkinson et al. 2000).

In conclusion, Polar Foods, Inc. has determined that the oxidative stability of cold pressed HiOmega ™ flaxseed oil is similar to other edible oils and the intended uses of HiOmega™ flaxseed oil is safe with respect to oxidative stability and lipid peroxidation.

Table 12. Effect on fatty acid profiles. Changes in fatty acid levels as a result of flaxseed oil supplementation. Each number refers to a reference listed at the bottom of the table which describes the subject, level of flaxseed oil supplementation and the context in which the fatty acid was measured. The n6:n3 ratio generally indicates the LA:ALA ratio of the flaxseed oil. Abbreviations: ALA: α-linolenic acid; LA: linoleic acid; DPA: docosapentanoic acid; EPA: eicosapentanoic acid; DHA: docosahexanoic acid; AA: arachidonic acid.

Fatty acid	Increased	Decreased	No Effect
ALA (18:3n-3)	1, 2a,b, 3a,b, 4,5b,6,7,8,9,10a,10b, 11,12a,b,13a,b,14b[i,ii,iv],14c[i,ii], 17b,c,d,e,f,18a[i,ii,iv,vi,viiiix],18b[i,vi,viii,ix], 20a,b, 21a,b,c,d, 23a,b, 24a,b,25,26a,b,27a,b,c,d,e,f,28,29a, 31,33a,d,34a,35a,b,40a,41a,42b,c,d ,43a,b,c,44a,45a,46a,b,48a,49a,50a, b,c,d,e,f,51a,53a,54a,55a,b,c,56a, 57b,c,d,e,f,58a,b,59a,b,60a,b,61a,b, 62a,b,c,d,63a,c,64a,b,d,e,66a,b,67b ,69a,70a,71a,72a,73a,b74a,75a,76a, b,c,d,e,77a,b,c,d,e,f,78a,b,c,79a,b,8 0a,82a,b,c,d,e,f,g,h,83a,b,c,d,e,f,g,h ,84a,b,85a,86a,87a,89a,b,c,90a,92a, b,c,d, 93a,94b,95a,96a,97a,b,c,d,98	57b	5a,15,17a,18a[iii,v,vii], 18b[ii,iii,iv,v,vii],27g,29b,3 3b,c,e,h,i,36a,42a,47a,b,c,5 7a,60c,64c,f,g,65a,67a,80b, 94a

Fatty acid	Increased	Decreased	No Effect
LA (18:2n-6)	1, 2b,12a,b, 14a[ii],14c[ii,iv], 19, 21a,b,d,35a,b,36a,41b,44a,46a,b,48a,49a,50c,51a,52a,57a,b,c,d,e,f,59a,b,60a,b,61a,61b,63a,b,c,71a,79a,b,82d,g,h83a,b,c,d,e,f,h,84b,86a,89c,92b,96a,98	2a,3a,4,6,14b[iii,iv],39a,40a,42a,b,c,47a,50b,d,e,f,53a,55a,b,c,56a,61b,62a,b,c,d,63a,c,69a,70a,73a,b,75a,76c,78a,b,c,80a,b,82a,b,c,e,f,84a,89a,b,92a	3b,5a,5b,7,9,10a,10b,11,13a,b,14a[i],14b[i,ii],14c[i,iii],15,17a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,18a[i,ii,iii,iv,v,vi,vii,viii],18b[i,ii,iii,iv,v,vi,vii,viii],21c,25,27a,b,c,d,e,f,g,41a,42d,43a,b,c,45a,47b,c,50a,58b,60c,64a,b,c,d,e,f,g,65a,66a,b,67a,b,72a,74a,76a,b,d,e,83g,85a,90a,92c,d,93a,94a,b,95a,97a,b
DPA (22:5n-3)	1,3b,4,5a,5b,9,10a,14a[i,ii,iii],14b[i,ii,iii,iv],14c[i,ii,iii,iv],19,21a,b,c,d,25,28,29a,33a,36a,38a,b,43b,c,45a,50c,f,51a,58a,b,59a,b,60a,b,c,64a,b,c,d,e,f,g,78a,b,c,80a,b,82a,b,c,d,e,f,g,h,89a,b,c,94a		3a,10b,11,42b,c,d,46a,b,47a,b,c,50a,b,d,e,65a,66a,b,85a,90a,94b

Fatty acid	Increased	Decreased	No Effect
EPA (20:5n-3)	1,2a,b,c,3,4,5a,5b,6,7,8,9,10a,12b,13a,b,14b[i,ii],14c[i,ii,iii,iv],15,17a,b,j,p,20a,b,21a,b,c,d,22a,23a,24a,b,c,d,25,26a,27a,b,c,d,e,28,29a,30c,e,f,33a,f,h,i,34a,35a,b,36a,37a,38a,b,39a,40a,41a,c,42a,b,43a,b,c,45a,46a,b,48a,49a,51a,52a,56a,57a,b,e,f,59a,b,60a,b,62a,b,c,d,63a,b,c,64a,b,c,d,e,f,g,65a,69a,70a,71a,74a,75a,76a,b,d,e,77a,b,c,d,e,78a,79a,b,80a,b,81a,c,d,82a,b,c,d,e,f,g,h,83b,c,e,g,84b,85a,86a,87a,89a,b,c,94a,95a,96a,97a,b,d,98	84a	3a,10b,11,12a,14a[i,iii],16,17c,d,e,f,g,h,i,k,l,m,n,o,q,r,18a[i,ii,iii,iv,v,vi,vii,viii,ix],18b[i,ii,iii,iv,v,vi,vii,viii,ix],27f,g,30a,b,d,33b,c,d,e,g,42c,d,47a,b,c,57c,d,58a,b,60c,66a,b,67a,b,72a,73a,76c,77f,78b,c,81b,82a,83a,d,f,h,90a,94b,97c
DHA (22:6n-3)	4,6,13a,14a[i,ii],14c[iii],19,21a,b,24a,b,c,d,29a,30b,c,f,31,32,33b,c,d,e,f,g,h,i,38a,39a,40a,42a,45a,46a,b,50c,f,52a,53a,54a,55b,c,56a,57a,b,c,58a,b,59a,b,60a,b,63b,64c,d,e,68b,c,69a,70a,73a,b,74a,75a,76a,b,d,e,77a,b,c,d,e,f,88a,89a,c,94a,96a,97a,b,d,98	11,12a,23a,35a,51a,80a,82a,b,c,d,e,f,g,h,83c,g,h,84a,b,85a,86a,87a	1,3a,b,5b,7,8,9,10a,10b,12b,13b,14a[iii,iv],14b[i,ii,iii,iv],14c[i,ii,iv],15,16,17a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,18a[i,ii,iii,iv,v,vi,vii,viii,ix],18b[i,ii,iii,iv,v,vi,vii,viii,ix],21c,d,30a,d,e,33a,35b,36a,38b,41c,42b,c,d,43a,b,c,47a,b,c,50a,b,d,e,60c,62a,b,c,d,63a,c,64f,g,66a,b,67a,b,71a,72a,76c,78a,b,c,80b,81a,b,c,d,83a,b,d,e,f,89b,90a,94b,95a,97c

Fatty acid	Increased	Decreased	No Effect
AA (20:4n-6)	17b,50c,57f	1,2c,6,10a,11,12a,14a[ii],14b[ii,iii],14c[i,ii,iii],16,17a,19,20a,b,21a,c,d,22a,23a,b,25,30a,b,c,f,33a,d,e,f,i,34a,35a,b,37a,39a,40a,41a,b,42a,43b,46a,b,47b,48a,49a,50f,52a,53a,54a,55a,b,c,56a,57a,b,c,59a,b,60a,b,c,61a,63a,b,c,64a,b,c,d,e,65a,68b,69a,70a,73b,74a,75a,76a,b,c,78a,b,c,79b,82a,b,c,d,e,f,g,h,83b,c,e,g,h,84a,b,85a,86a,89a,b,c,91a,92a,b,c,d,93a,94a,95a,97a,b,c,d	3a,b,4,5b,7,14a[i,iii,iv],14b[i,iv],14c[iv],15,17b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,18a[i,ii,iii,iv,v,vi,vii,viii,ix],18b[i,ii,iii,iv,v,vi,vii,viii,ix],21b,27a,b,c,d,e,f,g,30e,d,33b,c,g,h,36a,41c,42b,c,d,43a,47a,c,50a,b,d,e,51a,57d,e,58a,b,61b,62a,b,c,d,66a,b,67a,b,71a,72a,73a,76d,79a,80a,b,83a,d,f,90a,94b,98
Oleic (18:1n-9)	14b[i,ii,iv],14c[ii],48a,c,d,e,f,53a,56a,58a,93a,97a,b	6,13a,b,17f,21a,b,c,d,27a,b,c,d,e,30a,f,35a,b,43a,b,49a,50a,57a,b,d,e,f,58b,59a,61a,b,71a,95a	3a,b,4,5a,5b,6,7,11,14a[i,ii,iii,iv],14c[i],15,17a,b,c,d,e,g,h,i,j,k,l,m,n,o,p,q,r,23a,27f,g,30b,c,d,e,43c,50b,52a,54a,57c,59a,90a,92a,b,c,d,98
16:0		8,61a	27a,b,c,d,e,f,g,98
20:0			3a,b
22:0			3a,b
24:0			3a
18:3n-6		12a,b	
20:3n-6	35b	3a,6,21a	3b,5b,7,11, 21b,c,d,36a
22:4n-6		5a,7,11,14a[ii,iii],19, 21a,b,c,d	5b,14a[i],25
20:1n-9		21d	3b,7, 21b,c
24:1n-9			3a

4.1.29 References for Table 12: Fatty Acid Profiles

1. Healthy volunteers consuming dietary encapsulated flaxseed oil supplements (5.9 g/day ALA, 10.7 g/day flaxseed oil, n6:n3 ratio 0.27 on average) (Freese et al. 1997). Context: platelet lipids

2. Healthy volunteers consuming a diet supplemented with flaxseed oil (60 ml/day, 52.9% ALA = 31.74 ml ALA/day, n6:n3 ratio 0.39) (Budowski et al. 1984). Context: plasma lipids (a) triacylglycerol (b) cholesterol esters (c) phospholipids

3. Healthy volunteers consuming a diet supplemented with flaxseed oil (31.7 g/day, 21.28 % ALA =6.7 g ALA/day, n6:n3 ratio 0.72) (Kelley et al. 1993). Context: (a) serum lipids (b) peripheral blood mononuclear cells

4. Normotensive, mildly hypercholesterolemic volunteers consuming a diet supplemented with flaxseed oil (9.2 g ALA per serving, n6:n3 ratio 0.48) (Kestin et al. 1990). Context: plasma

5. Healthy, vegetarian volunteers consuming a diet supplemented with flaxseed oil (15.4 g ALA per day, n6:n3 ratio 1.0) (Li et al. 1999) Context: (a) platelet phospholipids (b) plasma phospholipid

6. Healthy volunteers consuming a diet supplemented with flaxseed oil (30 ml/day, 18.75 ml ALA/day, n6:n3 ratio 0.2) (Mest et al. 1983). Context: serum total phospholipids

7. Healthy volunteers consuming a diet supplemented with flaxseed oil (9.38 g ALA/day, n6:n3 ratio 0.33) (Sanders et al. 1983) Context: platelet phosphoglycerides

8. Normal, healthy volunteers expressing an atherogenic lipoprotein phenotype consuming a diet supplemented with flaxseed oil (30 g/day, 15 g ALA per day, n6:n3 ratio <1) (Wilkinson et al. 2005). Context: erythrocyte phospholipids

9. Overweight volunteers consuming a diet supplemented with flaxseed oil and flaxseed oil margarine (20 g ALA per day, n6:n3 ratio not stated) (Nestel et al. 1997). Context: plasma

10. Healthy volunteers consuming a diet supplemented with encapsulated flaxseed oil (3.51 g ALA/day n6:n3 ratio: 0.256) (Cao et al. 2006) Context: (a) erythrocyte membranes (b) plasma phospholipids

11. Rats consuming a diet supplemented with flaxseed oil (62% ALA, 1,2.5,5 % flaxseed oil wt/wt of diet, n6:n3 ratio 0.24) (Rupp et al. 1996) Context: thoracic aorta

12. Healthy volunteers consuming a diet supplemented with flaxseed oil (30 ml/day, 15.9 ml ALA, n6:n3 ratio 0.25) (Schwab et al. 2006) Context: (a) serum cholesteryl esters (b) serum triglycerides

13. Rats consuming a diet supplemented with flaxseed oil (20.3 % ALA, n6:n3 ratio 0.33) (Ramaprasad et al. 2005) Context: (a) liver (b) platelets

14. Rats consuming a diet supplemented with flaxseed oil (56.71 % ALA, n6:n3 ratio 0.39) (Barceló-Coblijn et al. 2005). Context: (a) brain (b) heart (c) liver [i] ethanolamine glycerophospholipid [ii] choline glycerophospholipid [iii] phosphatidylserine [iv] phosphatidylinositol

15. Healthy volunteers consuming a diet supplemented with encapsulated flaxseed oil (3.5 g ALA/day, n6:n3 ratio 0.36 of oil, n6:n3 of total diet 3.04) (Wallace et al. 2003). Context: plasma phospholipids

16. Healthy volunteers consuming a diet supplemented with encapsulated flaxseed oil (9 g/day, 4.0 g ALA/day, n6:n3 ratio: 0.37) (Healy et al. 2000). Context: neutrophil

17. Healthy volunteers consuming a diet supplemented with flaxseed oil (oil: 35 mg/kg body weight per day, 57.5 % ALA, n6:n3 ratio: 0.25 (for 70 kg subject this would be 4.3 g oil / day, 2.47 g ALA/day) (Layne et al. 1996). Context: (a) HDL triacylglycerol high P/S ratio diets (b) HDL triacylglycerol low P/S ratio diets (c) VLDL triacylglcerol high P/S (d) VLDL triacylglycerol low P/S (e) LDL triacylglycerol high P/S (f) LDL triacylglycerol low P/S (g) HDL cholesteryl ester high P/S ratio (h) HDL cholesteryl ester low P/S ratio (i) VLDL cholesteryl ester high P/S ratio (j) VLDL cholesteryl ester low P/S ratio (k) LDL cholesteryl ester high P/S ratio (l) LDL cholesteryl ester low P/S ratio (m) HDL plasma phospholipids high P/S (n) HDL plasma phospholipids low P/S (o) VLDL plasma phospholipids high P/S (p) VLDL plasma phospholipids low P/S (q) LDL plasma phospholipids high P/S (r) LDL plasma phospholipids low P/S

18. Patients with non-insulin-dependent diabetes mellitus consuming a diet supplemented with flaxseed oil (35 mg ALA/kg body weight/day) (Goh et al. 1997). Context: (a)[i] Plasma triacylglycerol low P/S ratio VLDL (a)[ii] LDL (a)[iii] HDL (a)[iv] Plasma phospholipids fraction low P/S ratio VLDL (a)[v], LDL (a)[vi] HDL (a)[vii] Plasma cholesteryl ester low P/S ratio VLDL (a)[viii] LDL (a)[ix] HDL (b)[i] Plasma triacylglycerol high P/S ratio VLDL (b)[ii] LDL (b)[iii] HDL (b)[iv] Plasma phospholipids fraction high P/S ratio

VLDL (b)[v] LDL (b)[vi] HDL (b)[vii] Plasma cholesteryl ester high P/S ratio VLDL (b)[viii]LDL (b)[ix] HDL

19. Rats consuming a diet supplemented with flaxseed oil (53.3 % ALA, n6:n3 ratio 0.39) (Al Makdessi et al. 1994) Context: heart sarcolemma phospholipids

20. Mice consuming a diet supplemented with flaxseed oil (49.7 % ALA, n6:n3 ratio 0.5) (Fritsche et al. 1989) Context: (a) splenocyte choline phosphoglyceride (b) splenocyte ethanolamine phosphoglyceride

21. Chicks consuming a diet supplemented with flaxseed oil (37.8 % ALA, n6:n3 ratio 0.75) (Fritsche et al. 1991) Context: (a) serum (b) splenocyte (c) bursa (d) thymus

22. Mice consuming a diet supplemented with flaxseed oil (80g/kg, 43.5 % ALA, n6:n3 ratio 0.32) (Hillyer et al. 2006) Context: (a) serum

23. Rats consuming a diet supplemented with flaxseed oil (48.8 % ALA, n6:n3 ratio 0.33) (Jeffery et al. 1996b). Context: (a) serum (b) lymphocyte

24. Chickens consuming a diet supplemented with flaxseed oil (34.6 % ALA, n6:n3 ratio of diet 0.85) (Anderson et al. 1989). Context: (a) egg yolks (b) serum of chicks hatched from eggs (c) brain tissue of chicks hatched from eggs (d) retinal fatty acids of chicks hatched from eggs

25. Dogs consuming a diet supplemented with flaxseed oil (20 g/day, 53 % ALA, n6:n3 ratio 0.33) (Anderson et al. 1994) Context: plasma lipids including phospholipids, triglycerides, free fatty acids, cholesterol esters

26. Mildly hypercholesterolemic men consuming a diet supplemented with flaxseed oil (9 g ALA/day, n6:n3 ratio 0.5) (Abbey et al. 1990). Context: (a) plasma fatty acids (b) HDL cholesteryl ester

27. Healthy female volunteers consuming a diet supplemented with ALA (Adam et al. 1986). Context: (a) LDL phosphatidyl choline (b) HDL phosphatidyl choline (c) plasma cholesteryl esters (d) LDL cholesteryl esters (e) HDL cholesteryl esters (f) platelet total lipids (g) HDL phosphatidylethanolamine

28. Healthy male volunteers consuming a diet supplemented with flaxseed oil (40 g oil/day, approximately 57 % ALA, n6:n3 ratio 0.28) (Allman et al. 1995). Context: platelet fatty acids

29. Healthy female volunteers consuming a diet supplemented with flaxseed oil (20 g oil/day, ALA 58.2 %, n6:n3 ratio 0.26) (Cunnane et al. 1993). Context: (a) plasma triglycerides (b) plasma phospholipids

30. Rats consuming a diet supplemented with flaxseed oil (diet: 13.8 %ALA, n6:n3 ratio 1.2) (Alsted 1992). Context: (a) brain phosphatidylinositole (b) brain phosphatidylserine (c) brain phosphatidylethanolamine (d) brain poly-phosphatidylinositol-4-phosphate PIP (e) brain phosphatidylinositol-4,5-bisphosphate PIP2 (f) heart phosphatidylethanolamine

31. Mice consuming a diet supplemented with flaxseed oil (oil not described) (Berger et al. 1992). Context: cardiolipins

32. N-3 deficient rats consuming a diet supplemented with flaxseed oil (1 ml/week, 53 % ALA, n6:n3 ratio not described) (Bicknell et al. 2002). Context: rod outer segment

33. Rats consuming a diet supplemented with flaxseed oil (10 % weight oil/weight diet, 58.6 % ALA, n6:n3 ratio 0.3) (Brown et al. 1984). Context: (a) breast milk (b) brain phosphatidylcholine (c) brain phosphatidylinositol/phosphatidylserine (d) brain diacyl phosphatidylethanolamine (e) brain plamalogen phosphatidylethanolamine (f) second generation brain phosphatidylcholine (g) second generation brain phosphatidylinositol/phosphatidyl serine (h) second generation brain diacyl phosphatidylethanolamine (i) second generation brain plamalogen phosphatidylethanolamine

34. Rats consuming a diet supplemented with flaxseed oil (7% oil, ALA not described, n3:n6 ratio not described, statistical significance not indicated) (Century et al. 1968). Context: (a) liver phospholipids

35. Pigs consuming a diet supplemented with flaxseed oil (5% oil/weight diet, 50.4 % ALA, n6:n3 ratio 0.36) (Chartrand et al. 2003). Context: (a) maternal blood plasma (b) endometrial tissues

36. Rats consuming a diet supplemented with flaxseed oil (20 % oil (99% flaxseed oil) 61.5% ALA, n3:n6 ratio 0.189) (Christiansen et al. 1991). Context: (a) liver phospholipids

37. Rats consuming a diet supplemented with flaxseed oil (10 % oil w/w diet, ALA 47 %, n6:n3 ratio 0.36) (Cleland et al. 1990). Context: (a) peritoneal exudates cell phospholipids, significance not noted

38. Rats consuming a diet supplemented with flaxseed oil (5% oil, 56.5 % ALA, n6:n3 ratio 0.28) (Cleland et al. 2005). Context: (a) heart phospholipids (b) plasma

39. Mice consuming a diet supplemented with flaxseed oil (10 % oil, 56 % ALA, n6:n3 ratio 0.27) (Cohen et al. 2005). Context: (a) serum

40. IL-10 knockout mice consuming a diet supplemented with flaxseed oil (10 % oil, 56 % ALA, n6:n3 ratio not described) (Cohen et al. 2005b). Context: (a) serum

41. Rats consuming a diet supplemented with flaxseed oil (5, 20 and 40 % energy, 50 % ALA, n6:n3 ratio 0.36) (Croft et al. 1984b). Context: (a) plasma phospholipids (b) kidney phospholipids (c) liver phosphatidylethanolamine

42. Rabbits consuming a diet supplemented with flaxseed oil (60 g/kg, 33.43 % ALA, n6:n3 ratio:0.53) (Vas Dias et al. 1982). Context: (a) platelet lipids (b) liver (c) adipose tissue (d) aortic lipids

43. Lactating women consuming a diet supplemented with flaxseed oil (20 g oil/day, 53.6 % ALA, n6:n3 ratio 0.28) (Francois et al. 2003). Context: (a) breast-milk fatty acid (b) maternal plasma fatty acid (c) maternal erythrocyte fatty acid

44. Lactating rats consuming a diet supplemented with flaxseed oil (10.2 % lipid in diet, 62.5 % ALA n6:n3 ratio not described) (Grigor et al. 1980). Context: (a) breastmilk fatty acid

45. For chronically ill volunteers consuming a diet supplemented with flaxseed oil (5.2 g oil/day, 58 % ALA, n6:n3 ratio 0.29) (Harper et al. 2006b). Context: (a) plasma fatty acid

46. Puppies consuming either a diet supplemented with flaxseed oil or breastmilk from mothers whose diets were supplemented with flaxseed oil (68.2 % ALA, n6:n3 ratio of diet 0.51) (Heinemann et al. 2005). Context: (a) suckling pups plasma phospholipids (b) weaned pups plasma phospholipids

47. Horses consuming a diet supplemented with flaxseed oil (8% oil, ALA content not described, n6:n3 ratio not described) (Henry et al. 1990). Context: (a) monocyte phosphatidylcholine (b) monocyte phosphatidylethanolamine (c) monocyte phosphatidylserine

48. Spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (62.5% ALA, n6:n3 ratio 0.21) (Hoffmann et al. 1986). Context: (a) kidney phospholipids

49. Female spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (14 % oil, 62.5 % ALA, n6:n3 ratio 0.21) (Hoffmann et al. 1985). Context: (a) kidney medulla phospholipids

50. Guinea pigs consuming a diet supplemented with flaxseed oil (5 % oil, 46.9 % ALA, n6:n3 ratio 0.45) (Huang et al. 1985). Context: (a) plasma cholesteryl

esters (b) plasma triglycerides (c) plasma total phospholipids (d) liver cholesteryl esters (e) liver triglycerides (f) liver total phospholipids

51. Renal ablated rats consuming a diet supplemented with flaxseed oil (15 % oil, ALA not described, n6:n3 ratio not described) (Ingram et al. 1995). Context: (a) renal tissue phospholipids

52. Children diagnosed with attention deficit hyperactivity disorder and consuming a diet supplemented with flaxseed oil (200 mg ALA/day, n6:n3 ratio not described) (Joshi et al. 2006). Context: (a) red blood cell phospholipids

53. Pups of rats consuming a diet supplemented with flaxseed oil while pregnant (7 % oil, 33% ALA, n6:n3 ratio 0.42) (Korotkova et al. 2004). Context: (a) pup serum phospholipids

54. Pups of rats consuming a diet supplemented with flaxseed oil while pregnant (7% oil, 33 % ALA, n6:n3 ratio 0.42) (Korotkova et al. 2005). Context: (a) pup serum phospholipids

55. Rats consuming a diet supplemented with flaxseed oil while pregnant (7% oil, 33 % ALA, n6:n3 ratio 0.42) (Korotkova et al. 2002). Context: (a) breastmilk (b) pup white adipose tissue total lipids (c) pup white adipose tissue phospholipids

56. Pups of rats consuming a diet supplemented with flaxseed oil (7 % oil, 33 % ALA, n6:n3 ratio 0.42) (Korotkova et al. 2004b). Context: (a) pup serum phospholipids

57. Rats consuming a diet supplemented with flaxseed oil (10 % oil, 58.4 % ALA, n6:n3 ratio 0.28) (Lee et al. 1988). Context: (a) liver phosphatidylcholine (b) serum phosphatidylcholine (c) aorta phosphatidylcholine (d) liver triglyceride (e) serum triglyceride (f) adipose tissue triglyceride *Note: ALA level in serum phophatidylcholine actually lower in flaxseed oil supplemented group than in palm oil supplemented group...anomalous!

58. Rats consuming a diet supplemented with flaxseed oil (0.48 % oil, 3.12 % omega-3 pufa, n6:n3 ratio not described) (Li et al. 2003). Context: (a) femoral cortical bone (b) femoral bone marrow

59. Rats exposed to carbon tetrachloride and consuming a diet supplemented with flaxseed oil (20 % oil, 50.7 % ALA, n6:n3 ratio 0.29) (MacDonald-Wicks et al. 2002). Context: (a) plasma (b) red blood cell membranes

60. Rats consuming a diet supplemented with flaxseed oil (10 % oil, 61.8% ALA, n6:n3 ratio 0.28) (Magrum et al. 1983). Context: (a) peritoneal macrophage

ethanolamine phosphoglyceride (b) peritoneal macrophage choline phosphoglyceride (c) peritoneal macrophage serine phosphoglyceride

61. Rats consuming a diet supplemented with flaxseed oil (3 % oil, 53 % ALA, n6:n3 ratio 0.26) (Mahoney et al.1983). Context: (a) renal fatty acid (b) aortic fatty acid

62. Volunteers consuming a diet supplemented with flaxseed oil (amount of oil not described, 55.6 % ALA, n6:n3 ratio 0.33) (Mantzioris et al. 1994). Context: (a) plasma phospholipids (b) plasma cholesteryl esters (c) plasma triglycerides (d) neutrophil phospholipids

63. Rats consuming a diet supplemented with flaxseed oil (10 % oil, 54.10 ALA, n6:n3 ratio 0.29) (Maranesi et al. 1988). Context: (a) plasma phospholipids (b) platelet phospholipids (c) aorta phospholipids

64. Rats consuming a diet supplemented with flaxseed oil (10 % oil, 61.8% ALA, n6:n3 ratio 0.28) (Marshall et al. 1983). Context: (a) splenocyte ethanolamine phosphoglyceride (b) splenocyte choline phosphoglyceride (c) thymocyte ethanolamine phosphoglyceride (d) mast cell ethanolamine phosphoglyceride (e) mast cell choline phosphoglyceride (f) peripheral lymphocyte ethanolamine phosphoglyceride (g) peripheral lymphocyte choline phosphoglyceride

65. Rats consuming a diet supplemented with flaxseed oil (10 % oil, 61.8 % ALA, n6:n3 ratio 0.28) (Marshall et al. 1982). Context: (a) liver ethanolamine phosphoglyceride

66. For patients accepted for elective cardiac surgery involving cardiopulmonary bypass and consuming a diet supplemented with flaxseed oil (10 ml/day, 58.7 % ALA, n6:n3 ratio 0.27) (Metcalf et al. 2007). Context: (a) atrial phospholipids (b) erythrocyte phospholipids

67. Dogs consuming a diet supplemented with flaxseed oil (200 mg/kg/day oil, 570 mg ALA, n6:n3 ratio 0.30) (Mueller et al. 2005). Context: (a) skin (b) plasma

68. Rats consuming a diet supplemented with flaxseed oil (10 % oil, 54.17 ALA, n6:n3 ratio 0.23) (Tarozzi et al. 1984). Context: (a) whole brain (b) optic nerve (c) visual cortex

69. Volunteers consuming a diet supplemented with flaxseed oil (30 ml oil/day, 62.5 % ALA, n6:n3 ratio 0.21) (Beitz et al. 1981). Context: (a) serum total phospholipids

70. Rats with established kidney disease consuming a diet supplemented with flaxseed oil (7% oil, ALA content not described, n6:n3 ratio not described) (Sankaran et al. 2007). Context: (a) kidney fatty acid

71. Pigs consuming a diet supplemented with flaxseed oil (5% oil, 56.9 % ALA, n6:n3 ratio 0.27) (Nguyen et al. 2004). Context: (a) adipose tissue

72. Patients with rheumatoid arthritis consuming a diet supplemented with flaxseed oil powder (30 g oil powder, 32 % ALA, n6:n3 ratio not described) (Nordström et al. 1995). Context: (a) serum

73. Polycystic kidney disease Han:SPRD-cy rats consuming a diet supplemented with flaxseed oil (5% oil, ALA 52.3 %, n6:n3 ratio 0.30) (Ogborn et al. 2002). Context: (a) kidney (b) liver

74. Male offspring of polycystic kidney disease Han:SPRD-cy rats consuming a diet supplemented with flaxseed oil (7% oil, ALA 52.3 %, n6:n3 ratio 0.30) (Ogborn et al. 2006). Context: (a) kidney

75. Female offspring of polycystic kidney disease Han:SPRD-cy rats consuming a diet supplemented with flaxseed oil (7% oil, ALA 52.3 %, n6:n3 ratio 0.30) (Ogborn et al. 2006). Context: (a) kidney

76. Rats consuming a diet supplemented with flaxseed oil (20.3 %ALA, n6:n3 ratio 0.33) (Ramaprasad et al. 2004). Context: (a) serum (b) liver (c) adipose tissue (d) heart (e) brain

77. Female rats consuming a diet supplemented with flaxseed oil (10 % oil, 53 % ALA, n6:n3 ratio 0.38) (Wiegand et al.1991). Context: (a) plasma total phospholipids (b) plasma triglycerides (c) plasma cholesterol ester (d) rod outer segment phosphatidylcholine (e) rod outer segment phosphatidylethanolamine (f) rod outer segment phosphatidylserine Note: AA levels are increased relative to coconut oil but decreased relative to safflower oil supplementation. LA levels are increased relative to coconut oil but decreased or unchanged relative to safflower oil supplementation.

78. Rats consuming a diet supplemented with flaxseed oil (15 % oil, 53 % ALA, n6:n3 ratio 0.28) (Dwivedi et al.2005). Context: (a) serum fatty acids (b) colon microsomal fraction (c) tumor microsomal fraction

79. Male volunteers with mild hypertension consuming a diet supplemented with flaxseed oil (60 ml/day, 64 % ALA) (Singer et al.1990b). Context: (a) serum triglycerides (b) serum cholesterol esters

80. Rats consuming a diet supplemented with flaxseed oil (20 % oil (99% flaxseed oil, 1 % sunflower oil), 62% ALA, n6:n3 ratio 0.19) (Rustan et al.1992). Context: (a) liver triacylglycerol (b) liver phospholipids

81. Vegan and omnivore volunteers consuming a diet supplemented with flaxseed oil (20 ml oil/day, 53.93 % ALA, n6:n3 ratio 0.34) (Sanders et al. 1981). Context: (a) vegan plasma choline phosphoglycerides (b) vegan platelet phosphoglycerides (c) omnivore plasma choline phosphoglycerides (d) omnivore platelet phosphoglycerides

82. Spontaneously hypertensive (SHR) and normotensive rats consuming a diet supplemented with flaxseed oil (15 % oil, 64 % ALA, n6:n3 ratio 0.23) (Singer et al. 1987). Context: (a) SHR liver triglycerides (b) SHR liver free fatty acids (c) SHR liver phosphatidylethanolamine (d) SHR liver phosphatidylcholine (e) normotensive liver triglycerides (f) normotensive liver free fatty acid (g) normotensive phosphatidylethanolamine (h) normotensive phosphatidylcholine

83. Volunteers consuming a diet supplemented with flaxseed oil (60 ml oil/day, 64 % ALA, n6:n3 ratio 0.23) (Singer et al.1986). Context: (a) normal volunteers serum triglycerides (b) normal volunteers serum cholesterol esters (c) normal volunteers serum phospholipids (d) hypertensive volunteers serum triglycerides (e) hypertensive volunteers serum cholesterol esters (f) hyperlipemic volunteers serum triglycerides (g) hyperlipemic volunteers serum cholesterol esters (h) hyperlipemic volunteers serum phospholipids

84. Rats consuming a diet supplemented with flaxseed oil (150 g oil/kg, 64 % ALA, n6:n3 ratio 0.23) (Singer et al. 1990c). Context: (a) spontaneously hypertensive rat renal medulla triglyceride (b) spontaneously hypertensive rat renal medulla free fatty acid

85. UVB irradiated mice consuming a diet supplemented with flaxseed oil (10% oil, 48.4 % ALA, n6:n3 ratio 0.46) (Takemura et al. 2002). Context: (a) dorsal skin total lipids

86. Pigs consuming a diet supplemented with flaxseed oil (10.5 % oil, 44 % ALA, n6:n3 ratio 0.41) (Turek et al.1996). Context: (a) alveolar macrophage

87. Normolipidemic male subjects consuming a diet supplemented with flaxseed oil (30 ml oil/day, 20 g ALA/day n6:n3 ratio of diet $\leq$ 1) (Wilkinson et al. 2000). Context: (a) erythrocytes

88. Rats reared in bright light and consuming a diet supplemented with flaxseed oil (10 % oil, 53 % ALA, n6:n3 ratio 0.38) (Wiegand et al.1995). Context: (a) rod outer segment

89. Rats consuming a diet supplemented with flaxseed oil (6.4 % oil, 34 % ALA in diet, 55 % ALA in oil, n6:n3 ratio 0.34 in diet) (Winters et al.1994). Context: (a) plasma phospholipids (b) erythrocyte phospholipids (c) liver microsomal phospholipids

90. Adults with ADHD consuming a diet supplemented with flaxseed oil (60 g oil/day, 59.6 % ALA, n6:n3 ratio not described) (Young et al. 2005). Context: (a) serum phospholipids

91. Mice consuming a diet supplemented with flaxseed oil (10 % oil, 57 % ALA, 0.28) (Zhang et al. 1996). Context: (a) lung

92. One kidney one clip rats consuming a diet supplemented with flaxseed oil (40 % of calories, 50.5 % ALA, n6:n3 ratio 0.36) (Codde et al. 1984). Context: (a) kidney total lipids (b) kidney phospholipids (c) aortic phospholipids (d) plasma phospholipids

93. Rats consuming a diet supplemented with flaxseed oil (20 % energy, 44 % ALA, n6:n3 ratio 0.5) as compared to safflower oil or hydrogenated coconut oil/safflower oil mixture (Codde et al.1984b). Context: (a) renal phospholipids

94. White rabbits consuming a diet supplemented with flaxseed oil (Bolton-Smith et al. 1984). Tissue (a) platelet total fatty acids (b) aortic total fatty acids

95. Spontaneously hypertensive rats consuming a diet supplemented with flaxseed oil (1 ml/day, 49.3 % ALA, n6:n3 ratio 0.33) (Sekine et al. 2007). Context: (a) liver total lipids

96. Overweight but otherwise healthy volunteers consuming a diet supplemented with flaxseed oil capsules (ALA 5 % of energy intake, 57 % ALA, n6:n3 ratio 0.32) (Nelson et al. 2007). Context: (a) erythrocyte cell membrane fatty acid

97. Rats consuming a diet supplemented with flaxseed oil (10 % oil, 53.5 % ALA, n6:n3 ratio 0.33) (Liautaud et al. 1991). Context: (a) phospholipid fraction of rat hearts (b) phospholipids fraction of cultured rat cardiomyocytes (c) non-phophorous lipid fraction of rat heart (d) non-phosphorous lipid fraction of cultured rat cardiomyocytes

98. Abdominally obese, sedentary but otherwise healthy volunteers consuming flaxseed oil capsules (~11 g ALA/day, 57 % ALA, n6:n3 ratio 0.32) (Nelson 2007b). Context: erythrocyte cell membrane fatty acids

4.2. SAFETY ASSESSMENT/GRAS DETERMINATION

4.2.1 Introduction

This section presents an assessment that the use of HiOmega™ flaxseed oil is safe and is also GRAS under the Federal Food, Drug, and Cosmetic Act (the Act) for direct addition to foods as a nutrient supplement at specified levels (Table 1).

4.2.2 Regulatory basis of GRAS Notification

The FDA regulations governing GRAS status in accordance with Sections 201(s) and 409 of the Act is set forth under 21 CFR 170.3 . This regulation states 'for a substance to be GRAS, the scientific data and information about the use of a substance must be widely known and there must be a consensus among qualified experts that those data and information establish that the substance is safe under the conditions of its intended use". "The general recognition of safety may be based only on the views of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (1) scientific procedures under 21 CFR 170.30(b)or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. under 21 CFR 170.30(c) and 170.3(f) (b) General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies which may be corroborated by unpublished studies and other data and information."

Furthermore, general recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food. This "common knowledge" element of a GRAS determination consists of two components: 1) the data and information relied upon to establish the scientific element of safety must be generally available; and 2) there must be a basis to conclude that there is a consensus among qualified expert about the safety of the substance for its intended use. .

4.2.3 General recognition of the safety of HiOmega™ flaxseed oil

This GRAS notification of the proposed uses and use level of HiOmega™ flaxseed oil is based upon the scientific procedures regulations as set forth under 21 CFR 170.3. The proposed uses and use level of HiOmega flaxseed oil is about 36 g/person/day which would be equivalent to about 25 g of ALA/person/day. Safety of HiOmega™ flaxseed oil is established in four steps. First, HiOmega™ flaxseed oil is shown to be essentially similar to regular, conventional flaxseed oil. Secondly, the safety of HiOmega™ flaxseed oil was established by a scientific review of the published, peer reviewed scientific literature regarding the physiological effects of dietary flaxseed oil. This literature review included a variety of levels of dietary flaxseed oil consumption with high levels of alpha linolenic acid consumption and a variety of omega6:omega3 ratios. The extensive literature review included effects on fatty acid profiles, bleeding time, glycemic control, cholesterol, triglycerides, blood pressure, eicosanoids, cardiovascular health, immune system, cancer, body weight, bone health, kidneys, liver, reproduction, eyes, skin, mental health, lungs, antibiotic, enzyme activity, blood, minerals

and vitamins, miscellaneous effects and oxidative stability. Thirdly, the estimated metabolic conversion of dietary ALA to EPA and DHA should not exceed the GRAS level of dietary intake of EPA and DHA. Fourthly, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

This safety was established by scientific review of the published, peer reviewed scientific literature regarding the physiological effects of dietary flaxseed oil consumption, by estimating potential human exposure to ALA from the intended uses of HiOmega™ flaxseed oil and by estimating the potential human exposure to EPA and DHA as a result of ALA metabolic conversion to EPA and DHA. HiOmega™ flaxseed oil is determined to be GRAS by demonstrating that the safety of this substance under its intended conditions of use is generally recognized among qualified scientific experts.

Determination of the safety and GRAS status of HiOmega™ flaxseed oil for direct addition to foods under the intended conditions of use has been made through the deliberations of Dr. Douglas M. Bibus PhD, Dr. Neil D. Ross PharmD, Dr. Robert Wildman PhD, RD and Dr. Richard F. Staack PhD, MBA. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, and have concluded:

> Polar Foods Inc. Hiomega flaxeed oil is GRAS for addition to foods. No evidence exists in the available information on flaxseed oil that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public health when

flaxseed oil is used at the proposed intended use level of Polar Foods Inc. HiOmega flaxseed oil.

It is their opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same scientific conclusion. Therefore, HiOmega™ flaxseed oil is safe and is GRAS for the proposed uses in Table 1. Because Polar Foods, Inc. HiOmega™ flaxseed oil is GRAS for it's proposed uses, it is excluded from the definition of a food additive, and thus may be lawfully marketed and sold for these uses in the U.S. without promulgation of a food additive regulation under 21 CFR.

Therefore, it is concluded, based on scientific procedures, that the intended use HiOmega™ flaxseed oil as shown in Table 1, is safe and is also GRAS.

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Polar Foods

SUMMARY AND CONCLUSION OF EXPERT PANEL

We, the Expert Panel, independently and critically evaluated an extensive volume of available scientific, peer reviewed literature regarding the safety, toxicity and health effects of flaxseed oil. The results of this body of literature, including human dietary intake and animal (rats, mice, cats, rabbits, hamsters, dogs and chimpanzees) dietary intake studies did not indicate any consistent adverse effects of flaxseed oil due either to flaxseed oil itself, ALA or the metabolic products EPA and DHA. Similarly, this body of literature did not indicate any consistent adverse effects which were dose dependent or dependent upon omega 6:omega 3 ratios. Historically, flaxseed and flaxseed oil has been part of the world diet for millennia.

In a number of human studies, beneficial effects of flaxseed oil dietary consumption are noted. Human and/or animal studies indicate flaxseed oil supplementation may lower triglyceride levels, reduce blood pressure, decrease inflammatory eicosanoid production, have antithrombotic and antiarrythmic effects, reduce the growth and/or metastases of certain cancers, have a protective effect on bone metabolism, reduce renal injury associated with chronic kidney disease, maintain retina and brain DHA levels, improve skin health and improve mental health.

The Panel notes that extensive research continues on the potentially beneficial effects of flaxseed oil, ALA and flaxseed lignans particularly in the areas anti-cancer effects, kidney disease, cardiovascular effects, metabolism to EPA and DHA and effect of omega 6:omega 3 ratios in the typical North American diet.

In summary, we, the Expert Panel, have critically evaluated the information summarized in this report and conclude that, based upon scientific procedures, all natural, cold pressed HiOmega flaxseed oil, which contains 70 % alpha-linolenic acid, a low saturated fat level and a beneficial omega 6:omega 3 ratio, produced in accordance with current good manufacturing practices (21 CFR 182.1(b)), to be generally recognized as safe (GRAS) for use as replacement for other vegetable oils at a level of 36 g per person per day (25 g ALA per person per day) as set forth in Table 1.

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