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January 20, 2006

Robert L. Martin, Ph.D.
Deputy Director
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Premarket Approval
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Bob:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), Flax Canada 2015, of Winnipeg, Canada, through me as its agent, herby provides notice of a claim that the use of whole and milled flaxseed described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because Flax Canada 2015 has determined that the intended addition of whole and milled flaxseed to conventional foods, including meat and poultry products, is generally recognized as safe (GRAS) based on scientific procedures.

As required, three copies of the notification are provided for FDA's use, and a fourth copy for the use of USDA's Food Safety and Inspection Service. Additionally, four copies are provided of the Conclusion of the Expert Panel, including the signatures of the four members of the panel.

If you have any questions regarding this notification, please feel free to contact me at 804-742-5548 or jh@jheimbach.com.

Sincerely,

James T. Heimbach, Ph.D., F.A.C.N.
President

Encl.

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Determination of the GRAS Status of the Addition of Whole and Milled Flaxseed to Conventional Foods and Meat and Poultry Products

Prepared for
Flax Canada 2015 Inc.

Prepared by
JHeimbach LLC
Port Royal VA

January 2009

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1. GRAS EXEMPTION CLAIM

Flax Canada 2015 Inc., through its agent JHEIMBACH LLC, hereby notifies the Food and Drug Administration that the uses of whole and milled flaxseed described below are exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Flax Canada 2015 Inc. has determined through scientific procedures that such uses are generally recognized as safe (GRAS).

1/16/09

James T. Heimbach, Ph.D., F.A.C.N.
President, JHEIMBACH LLC

Date

1.1. Name and Address of Notifier

Flax Canada 2015
465—167 Lombard Avenue
Winnipeg, Manitoba R3B 0T6
Canada

Contact:	Kelley C. Fitzpatrick
Telephone:	204-982-2115
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1.2. Name of GRAS Substance

The common name of the substances that are the subject of this GRAS notice are whole flaxseed and milled flaxseed. Flaxseed is also known as linseed, especially in Europe; in North America the term "linseed" is more often applied to non-food uses of flaxseed plants and their oil. Flaxseed is also called simply flax, and milled flaxseed is also referred to as ground flaxseed or crushed flaxseed as well as milled flax, ground flax, or crushed flax.

1.3. Intended Use and Consumer Exposure

Whole and milled flaxseed are intended to be added to conventional foods as sources of α-linolenic acid and functional fiber to increase the daily intake of essential n-3 fatty acids and total dietary fiber. Flaxseed is also intended be added to foods, including meat and poultry products, as binders. Flaxseed may also provide additional technical effects such as firming, texturizing, stabilizing, bulking, and thickening.

1.4. Basis for GRAS Determination

Although flaxseed has a long history of consumption in the United States, as well as in Canada and other countries, Flax Canada's GRAS determination for the intended uses of whole and milled flaxseed is based on scientific procedures as described under 21 CFR §170.30(b). Comprehensive searches of the literature through May 2008 were conducted by JHeimbach LLC and served as the basis for preparation of a monograph summarizing the totality of the available information germane to determining the safety of the intended uses of whole and milled flaxseed.

It may be concluded that whole and milled flaxseed are safe under their intended conditions of use because the amounts of α-linolenic acid and dietary fiber provided by these uses are within recommended levels and because the total exposure to flaxseed and its constituents resulting from these uses is well within levels shown by extensive animal research and human studies to be safe and tolerable. An Expert Panel determined the intended use of whole and milled flaxseed to be safe, and also GRAS, by demonstrating that the safety of this level of intake is based on publicly available and accepted information and is generally recognized by experts qualified by scientific training and experience to evaluate the safety of substances added to food.

Therefore, the intended uses of whole and milled flaxseed are determined to be safe and GRAS. Determination of the safety and GRAS status of whole and milled flaxseed for direct addition to food under their intended conditions of use was made through the deliberations of an Expert Panel consisting of Joseph F. Borzelleca, Ph.D., Walter H. Glinsmann, M.D., Robert J. Nicolosi, Ph.D., and John A. Thomas, Ph.D., who reviewed the information in this monograph as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. They critically reviewed and evaluated the publicly available information, including the potential human exposure to flaxseed and intake of α-linolenic acid, dietary fiber, and other constituents resulting from the intended use of whole and milled flaxseed, and individually and collectively concluded that the available information on flaxseed contains no evidence that demonstrates or suggests reasonable grounds to suspect a hazard to the public health under the intended conditions of use of whole and milled flaxseed.

It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data would reach the same conclusion. Therefore, whole and milled flaxseed are GRAS by scientific procedures under the conditions of use described.

1.5. Availability of Information

The data and information that serve as the basis for the GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of James T. Heimbach, Ph.D., President, JHEIMBACH LLC, 923 Water Street, P.O. Box 66, Port Royal VA 22535, telephone 804-742-5548 and e-mail jh@jheimbach.com.

2. IDENTITY OF THE SUBSTANCE

2.1. Substance Name

The names of the substances are whole flaxseed and milled flaxseed.

2.2. Trade or Common Names

Flaxseed is sold under many brand names, but the generic product is known as flaxseed or simply flax. The term flax is also used to refer to the entire plant. It may also be referred to as linseed, especially in Europe, but in Canada and the U.S. the term linseed is usually reserved for industrial products (such as boiled linseed oil) from the flax plant. Milled flaxseed is also called ground flaxseed and crushed flaxseed, or milled flax, ground flax, and crushed flax.

2.3. Botanical Identification

The taxonomic identity of flax is *Linum usitatissimum* L. *Linum* is the largest genus within the flax family Linaceae (Diederichsen and Richards 2003), which is a family of herbaceous or shrubby dicotyledonous plants in the order Linales, characterized by mostly capsular fruit, stipulate leaves, and exappendiculate petals. The genus *Linum* is divided into 6 sections, some of which are wild-growing and many of which are tropical or semi-tropical, but the species *usitatissiumu* is a member of section *Linum*, which is characterized by large flowers, elongate fruiting pedicels, and alternating leaves without stipular glands and glabrous. *L. usitatissimum* does not occur as a wild plant and is grown primarily in temperate climates; it is likely descended from wild progenitors in the area around the Mediterranean and extending into northern temperate climates.

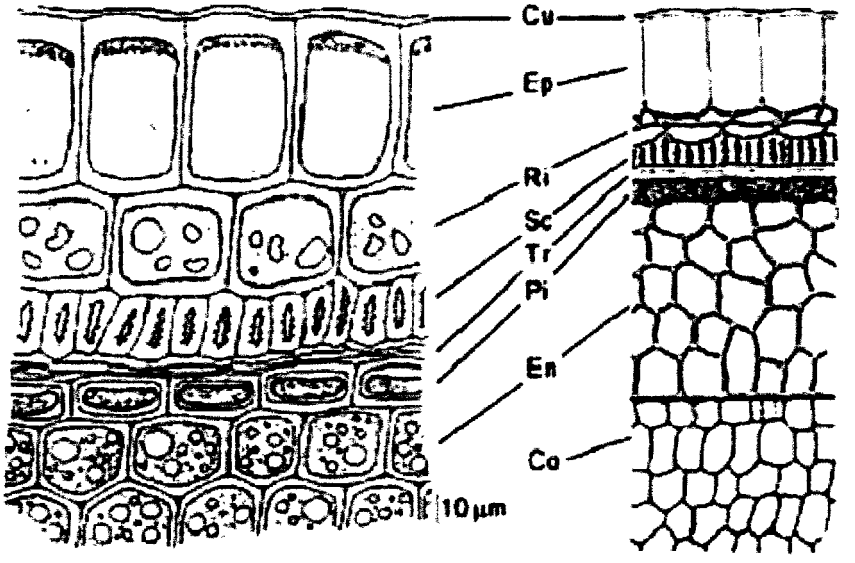
2.4. Description of Flaxseed

Flax is a summer crop grown in temperate climates (Diederichsen and Richards 2003). According to Hall et al. (2006), it is grown in about 50 countries, mostly in the northern hemisphere, with Canada as the largest producer accounting for about 33% of world production with 42 million bushels in 2004 (Hall et al. 2006). U.S. flaxseed production amounted to about 6 million bushels in 2007, with over 90% being produced in North Dakota (AmeriFlax 2008).

About 12 to 16 weeks elapses from the time of sowing until the end of flowering and the development of bolls containing seeds, when the seeds may be harvested (Diederichsen and Richards 2003). Flax plants range from less than 0.5 m to about 1 m in height. All branches of the main shoot terminate in flowers, which are most frequently blue or white. Each flower forms 10 capsules which may contain up to ten seeds each. The seeds are ovoid and about 3.5 to 5.0 mm in length, weighing about 5 to 12 mg apiece. The color of the seeds ranges from yellow to dark brown with a shiny surface. While the colors of specific crops of flaxseed may be extolled

in marketing messages, there is no significant nutritional or safety related difference between flaxseeds of different colors.

The cross section of flaxseed is shown below in Figure 1 (from Diederichsen and Richards 2003). About 75% of the flaxseed oil (mainly as triacylglycerols) is stored in the cotyledons, 22% in the seed coat, and 3% in the embryo or germ (Daun et al. 2003).



Cross-section of flaxseed. Cu: cuticula; Ep: epidermis; Ri: ring-cells; Sc: sclerenchyma; Tr: transversal-cells; Pi: pigment-cells; En: endosperm; Co: cotyledon

Figure 1. Cross-Section of Flaxseed (from Diederichsen and Richards 2003).

2.5. Flax Cultivars

Varieties of flax grown in Canada and the U.S. include AC Linora, Neche, AC McDuff, Omega, Verne, Linola™ 947, Linola™ 989, Linola™ 1084, Linola™ 2047, Linola™ 2090, Linola™ 2126, Linola™ 2149, AC Emerson, Prompt, Day, CDC Normandy, CDC Triffid, CDC Valour, AC Watson, AC Carnduff, CDC Arras, CDC Bethune, Taurus, Cathay, Pembina, Hanley, Lightning, CDC Mons, Macbeth, Prairie Blue, CDC Gold, Necoma, York, CDC Sorrel, Prairie Thunder, and Prairie Grande. This is not intended to be an exhaustive listing of the cultivars producing food-grade flaxseed addressed by this GRAS determination. Development of new varieties is an ongoing process with several new cultivars appearing in Canadian production each year (Daun et al. 2003).

2.6. Harvesting of Flax

Flax plants follow a life cycle consisting of 3 parts (Flax Council 2008):

- 45- to 60-day vegetative period
- 15- to 25-day flowering period
- 30- to 40-day maturation period

The length of these periods may vary due to changes in hydration, environmental temperature, or disease. The mature fruit of the flax plant is a dry boll or capsule that contains the seeds and slightly opens at the apex when ripe without allowing seeds to fall out. The crop is considered to be fully mature when 75% of the bolls have turned brown.

Harvesting flax crops is conducted either by straight combining (the preferred method if the crop is completely dry) or cutting with a swather followed by threshing (Flax Council 2008). Typically, crops are harvested for threshing if the moisture content is 10% or less, but may be harvested at higher moisture contents and dried using specialized facilities.

2.7. Composition of Flaxseed

2.7.1. Introduction

The epidermal cells of the flaxseed produce mucilage, composed of neutral and acidic polysaccharides, polypeptides, and glycoproteins (Bhatty 1993; Hall et al. 2006). Dietary fiber is about 28% of the flaxseed and protein is about 10% to 31%, average 20% (Hall et al. 2006). Flaxseed also contains about 35-45% oil (Hall et al. 2006; Wanasundara et al. 1999), about 52% α-linolenic acid (ALA; range 50% to 62%). The oil content depends on location, cultivar, and environmental conditions. Plant lignans are present at up to about 13 mg/g of the seed.

It is important to note that interpretation of figures regarding composition is difficult if the data are not accompanied by details of the source of the samples tested as well as analytical procedures utilized in testing (Daun et al. 2003). Variation in sample source and analytical techniques can result in substantial differences in values, and these uncertainties may be found even in authoritative datasets produced under government auspices.

2.7.2. Proximates

According to Release 20 of the U.S. National Nutrient Databank for Standard Reference (USDA/ARS 2007), the proximate content of flaxseed (*Linum usitatissimum*)—based on analyses of only 3 samples for most nutrients—is as shown in Table 1. Neither the origin of the flaxseed samples analyzed nor the analytical methods were described.

Table 1. Proximate Content of Flaxseed (USDA/ARS).

Nutrient	Unit	Content (per 100 g)
Water	g	6.96
Energy	kcal	534
Protein	g	18.29
Total lipid	g	42.16
Ash	g	3.72
Carbohydrate	g	28.88
Total dietary fiber	g	27.30
Total sugars	g	1.55
Sucrose	g	1.15
Glucose	g	0.40
Fructose	g	0.00
Lactose	g	0.00
Maltose	g	0.00
Galactose	g	0.00
Source: USDA/ARS (2007)		

Tables of the nutrient content of Canadian foods were prepared by Health Canada (1999a); the proximate analysis offered for flaxseed is shown below in Table 2. As with the USDA data, information was not provided regarding sources or analytical methods.

Table 2. Proximate Content of Flaxseed (Health Canada).

Nutrient[1]	Unit	Content (per 100 g)
Energy	kcal	495
Protein	g	18
Total lipid	g	34[2]
Carbohydrate	g	37
Dietary Fiber	g	17

[1]Values are given for the weight of the edible portion as described (i.e., flaxseeds)

[2]This value may be low due to use of an inappropriate analytical method; see discussion on page 8

Source: Health Canada (1999a)

Analyses of flaxseed grown in western Canada conducted in 2007 by the Canadian Grain Commission (DeClercq 2008; Table 3), resulted in similar values, although slightly higher in both protein and lipids than either the USDA/ARS (2007) or Health Canada (1999a) data. The reported proximate content of flaxseed grown between 1997 and 2006 is shown in Table 3, below.

Table 3. Flaxseed, No. 1 Canada Western Quality Data and Fatty Acid Composition for 2007 Harvest Survey.

Parameter	Year		
	2007	2006	1997-2006 Mean
Oil content (%[1])	44.7	45.9	44.7
Protein content (%[1,2])	24.3	23.6	23.2
Free fatty acids (%)	0.16	0.16	0.22
Iodine value	183.5	189.8	192.7
Linolenic acid (% in oil)	52.6	55.8	57.7
Oleic acid (% in oil)	20.6	19.5	18.2
Linoleic acid (% in oil)	16.2	15.6	15.1
Palmitic (% in oil)	5.0	5.0	5.2
Stearic (% in oil)	3.6	3.6	3.4

[1] Dry matter basis
[2] Nitrogen content x 6.25 (this value used to give an estimate of crude protein)
Source: DeClercq (2007)

Similar results were reported in a Flax Co-Operative Test Report conducted for the Prairie Recommending Committee for Oilseeds over 2005-2007 with various cultivars (2 check varieties and 26 experimental lines) throughout Canada (Duguid 2007). Oil content ranged from 45.3 to 48.9% with a mean of 46.8%. Protein content (determined by near-infrared measurements and expressed as N x 6.25) was reported to range from 23.4 to 25.7% with a mean of 24.8% for seed and from 43.9 to 48.2% with a mean of 46.5% for meal. Iodine number (calculated from fatty acid composition) was reported to vary from 190.4 to 199.9 with a mean of 194.3.

2.7.3. Lipids*

Oomah and Mazza (1993) analyzed the oil content (on a dry weight basis) of 11 flaxseed cultivars grown in Canada in 1987, 1988, and 1989. The results, shown in Table 4 on the following page, demonstrate that there is little year-to-year variability in the oil content of flaxseed. These data are somewhat closer to the USDA/ARS (2007) value (wet weight basis) for total lipid than are those of Health Canada (1999a) (wet weight basis) or DeClercq (2007) (dry weight basis).

Variations in analytical methodology for oil content can result in variances of up to 10% (Daun et al. 2003; Daun and Thomas 2005). Since extraction of oil from flaxseed is difficult, typical methods used for determining oils in food often result in low values. The Health Canada (1999a) value of 34% is a good example of this problem; the laboratories apparently employed AOAC Official Method 933.05 (Fat in Cheese), which can underestimate the lipid content of oilseeds (Daun and Thomas 2005). Other factors affecting the analysis of flaxseed oil content include level of extraction with neutral solvents, fineness of grind of flaxseed, and number of extractions. For determination of a nutritional fat value, the neutral lipid obtained from extraction with neutral solvents (3 extractions to ensure complete removal of neutral lipid) can be transesterified and the methyl ester content identified by gas chromatography (House et al. 1994).

The reported oil content of Canadian commercially grown flaxseed averaged 44% (range of 35-50%) during 1998-2002 (Daun et al. 2003). Processing and combining of flaxseed for export tightened the reported range of oil content to 42.3-45.8%.

Analysis of lipid fractions (Daun et al. 2003) showed the main components to be triacylglycerols (36-40.7%); monoacylglycerols (0.5-0.9%); diacylglycerols (0.9-2.6%); free fatty acids (0.1-3.4%); sterols, sterol esters, and hydrocarbons (0.5-2.9%); total neutral lipids (38.6-43.4%); complex lipids (0.4-1.8%); glycolipids (1.0-2.9%); and phospholipids (0.1-2.3%). Other components included tocopherols (0.0088-0.0572%) and various pigments such as carotenes, lutein, and chlorophyll.

---

* Daun et al. (2003) provide the following description of the lipid/fat/oil terminology in reference to flaxseed:

"Nutritional and labeling guidelines use the term fat to refer to neutral lipids from any source, and the nutritional and labeling definition of fat referes to the amound ot triacylglycerol that would be made up from the fatty acids derived from all of the acyl lipids in a food. For oilseeds used for processing to provide oil and meal, the term oil content referes to those neutral lipids obtained by exhaustive extraction with a non-polar lipid solvent, usually hexane or a related mixture of hydrocarbons. This mixture is composed almost exclusively of triacylglycerols and provides a good estimate of the nutritional fat content although it fails to account for a small amount of polar lipid, mainly from phospholipids which might make up as much as 2% of the total mass of flax seeds."

Table 4. Oil Content of Flaxseed Cultivars.

Cultivar	Oil (%; dry-weight basis)		
	1987	1988	1999
AC Linora	NA[1]	43.0	43.9
Andro	43.2	41.8	42.6
Dufferin	44.8	42.8	NA[1]
Flanders	44.7	42.9	43.7
Linott	43.2	42.0	43.2
McGregor	43.9	41.8	42.4
Noralta	42.1	40.5	41.0
NorLin	43.6	41.8	42.2
NorMan	44.6	42.7	43.3
Somme	43.5	42.1	42.7
Vimy	44.6	43.0	43.9

[1]NA = data not available
Source: Oomah and Mazza (1993)

2.7.3.1. Fatty Acids

Typically, flaxseed contains α-linolenic acid (ALA) at >50% of total fatty acids, palmitic acid at ~5% of total fatty acids, stearic acid at ~3% of total fatty acids, oleic acid at ~18% of total fatty acids, and linoleic acid at ~14% of total fatty acids (Daun et al. 2003). Flaxseed oil also contains about 1% of other fatty acids with chain lengths up to C_{24}. Flaxseed oil is a common unsaturated oil with the degree of unsaturation correlated with levels of ALA but negatively correlated with levels of oleic, linoleic, and saturated fatty acids (Eckey 1954). The degree of unsaturation is also affected by the flaxseed variety and growing conditions (Canvin 1965; Dybing and Zimmerman 1965, 1966; Daun et al. 2003).

Bean and Leeson (2002) analyzed the fatty acid content of 23 samples of flaxseed collected from commercial feed mills in Ontario (which may include seed grown all over Canada), presenting the data as percentages of total fatty acids. Their results, summarized in Table 5, show that α-linolenic acid (ALA) constitutes more than half of the total fatty acid content of flaxseed, with most of the remainder being oleic or linoleic acid.

Table 5. Fatty Acid Distribution of Canadian Flaxseed.

Fatty Acid	Percentage of Total Fatty Acids			
	Mean	Standard Deviation	Minimum	Maximum
α-Linolenic (C18:3 n-3)	57.11	1.68	51.52	59.42
Oleic (C18:1)	18.50	1.86	16.12	25.13
Linoleic (C18:2 n-6)	14.44	0.80	12.94	15.23
Palmitic (C16:0)	5.61	0.19	5.15	5.90
Stearic (C18:0)	3.15	0.33	2.69	4.10
Palmitoleic (C16:1)	0.06	0.01	0.04	0.09
Source: Bean and Leeson (2002)				

The USDA/ARS (2007) data also include estimates of the fatty acid content of flaxseed based on from 1 to 7 samples, expressed as percentages of the total flaxseed. These values are shown below in Table 6. It can be seen that 72% of the fatty acids in flaxseed are polyunsaturated and 80% of the polyunsaturates are α-linolenic acid.

Table 6. Fatty Acid Content of Flaxseed.

Fatty Acid[1]	Content (g/100 g flaxseed)
Total saturates	**3.663**
14:0	0.008
15:0	0.005
16:0	2.165
17:0	0.018
18:0	1.330
20:0	0.052
22:0	0.052
24:0	0.031
Total monounsaturates	**7.527**
16:1 undifferentiated	0.024
18:1 undifferentiated	7.359
20:1	0.067
22:1 undifferentiated	0.013
24.1 *cis*	0.064
Total polyunsaturates	**28.730**
18:2 undifferentiated	5.903
18:3 undifferentiated	22.813
20:2 n-6 *cis, cis*	0.007

[1]Fatty acids not detected are omitted.
Source: USDA/ARS (2007)

Similar results were reported with various cultivars (2 check varieties and 26 experimental lines) throughout Canada (Duguid 2007). Fatty acid composition (determined by gas liquid chromatography) was reported to range as follows:

- 16:0—4.5 to 6.2% (mean = 5.3%)
- 18:0—2.7-4.1% (mean = 3.4%)
- 18:1—14.8-19.6% (mean = 17.4%)
- 18:2—13.6-18.2% (mean = 15.8%)
- 18:3—55.5-60.1% (mean = 58.1%)

Variation in oil content and fatty acid values as high as 10% have been reported using different analytical methodologies and sample type (e.g., wet versus dry basis) (Daun and Thomas 2005), as shown in Table 7 below:

Table 7. Variation in Oil Content and Fatty Acid Values By Sample Type and Analytical Method

Parameter	Ground Flaxseed				Whole Flaxseed			
	Sample "as is"		Dry Matter Basis		Sample "as is"		Dry Matter Basis	
	GRL[1]	CL[2]	GRL	CL	GRL	CL	GRL	CL
Nitrogen[3] (%)	3.6	3.5	3.9	3.8	4.1	4.0	4.4	4.4
Fat[4] (%)	41.1	35.7	44.6	39.2	36.8	28.9	40.3	31.7
Moisture[5] (%)	7.8	8.8	0	0	8.7	9.1	0	0
FATTY ACIDS[6] (g/100g)								
C14:0	ND	ND	ND	ND	ND	ND	ND	ND
C16:0	1.9	1.5	2.1	1.6	1.7	1.3	1.8	1.4
C16:1	Trace	1.5	Trace	ND	Trace	ND	Trace	ND
C18:0	1.1	0.9	1.2	1.0	1.3	1.0	1.4	1.1
C18:1	7.3	5.5	7.9	6.1	7.8	5.7	8.5	6.3
C18:2	5.3	4.1	5.8	4.5	5.2	3.8	5.7	4.1
C18:3	23.1	17.1	25.1	18.9	18.7	13.3	20.5	14.6
C20:0	Trace	Trace	0.1	Trace	Trace	Trace	0.1	Trace
C20:1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
C20:2	ND	ND	ND	ND	ND	ND	ND	ND
C20:3	ND	ND	ND	ND	ND	ND	ND	ND
C20:4	ND	ND	ND	ND	ND	ND	ND	ND
C22:0	Trace	ND	Trace	ND	Trace	ND	0.1	ND
C22:1	ND	ND	ND	ND	ND	ND	ND	ND
C22:2	ND	ND	ND	ND	ND	ND	ND	ND
C24:0	Trace	ND	Trace	ND	Trace	ND	Trace	ND
C24:1	0.1	ND	0.1	ND	0.1	ND	0.1	ND
Others	0.3	ND	0.3	ND	0.2	ND	0.3	ND
Total Fatty Acids	39.1	29.1	42.4	31.9	35.0	25.1	38.3	27.6

[1]Grain Research Laboratory
[2]Commercial Laboratory
[3]GRL used AOCS Dumas Combustion Method Ba 4e-93; CL used AOAC Kjeldahl Method 991.20
[4]GRL used AOCS Method Am 2-93; CL used AOAC method 933.05
[5]GRL used ISO forced air oven method 665; CL used AOAC vacuum oven method 964.22
[6]GRL used AOCS Method Ce 1-62; CL used AOCS Ce 1-62
ND=not detected
Source: Daun and Thomas (2005)

2.7.3.2. Triacylglycerols and Free Fatty Acids

According to research by Wanasundara et al. (1999), 96% of the lipids in flaxseed are present in neutral form, primarily triacylglycerols with a small presence of free fatty acids. Polar lipids (glycolipids and phospholipids) account for 1.4%, and the major phospholipid is phosphatidylcholine. As a percentage of the total flaxseed, the neutral lipids were present in the concentrations shown in Table 8.

Table 8. Neutral Lipid Content of Flaxseed.

Neutral Lipid	Concentration (mg/100 g Fresh Seed)
Monoacylglycerol	0.34
Diacylglycerol	0.64
Triacylglycerol	29.60
Free fatty acids	0.42
Sterols	trace
Source Wanasundara et al. (1999)	

Hall et al. (2006) reported the following average distributions of triacylglycerols (Table 9). The most prevalent triacylglycerol is composed of 3 linolenic acid chains; this is followed by triacylglycerols with 2 linolenic acids and then by those with a single linolenic acid. The most prevalent triacylglycerol that does not contain linolenic acid is present at only 1.1%.

Table 9. Triacylglycerols in Flaxseed.

Triacylglycerol	Percent
LnLnLn	30.4
LaLnLn	18.7
OLnLn	13.5
LnLnP	6.9
OLaLn	5.9
LaLaLn	5.3
OLnO	4.2
SLnLn	4.1
OLnP	3.1
LnLaP	3.0
SLaLa	1.1
OLaLa	1.0
LaLaLa	0.9
OLaO	0.8
LaOP	0.6
PLnP	0.5
La=linoleic acid; Ln=linolenic acid O=oleic acid; P=palmitic acid S=stearic acid	
Source: Hall et al. (2006)	

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2.7.3.3. α-Linolenic Acid

As cited in Bean and Leeson (2002), α-linolenic acid (ALA) constitutes 57% of the lipid content of flaxseed or about 25% of the whole seed. ALA is an 18-carbon fatty acid with 3 double bonds, all in *cis* formation, at carbons 9, 12, and 15. The systematic name of ALA is 9, 12, 15-octadecatrienoic acid, and it has CAS Registry number 463-40-1. Because these 3 *cis* double-bonds introduce a pronounced bend in the carbon chain, ALA has a low melting point of -16.5°C as compared to -8.5°C for linoleic acid. Although the carbon atoms in carboxylic acids are officially numbered from the carboxyl end of the chain, fatty-acid nomenclature numbers them from the methyl end. Since the first double bond occurs at the 3rd carbon from the methyl end, ALA is an n-3 fatty acid, often referred to as an "omega-3 fatty acid." With chemical formula $C_{18}H_{30}O_2$ or, more meaningfully, $CH_3(CH_2CH{=}CH)_3(CH_2)_7COOH$, the molecular weight of ALA is 278.433. The chemical structure of ALA is shown in Figure 2, below.

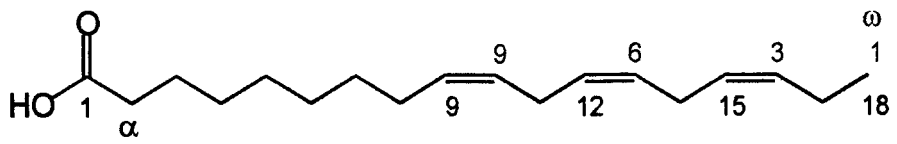

Figure 2. Chemical Structure α-Linolenic Acid.

ALA is regarded as an essential fatty acid. Humans cannot synthesize this fatty acid and a lack of it results in adverse clinical symptoms including neurological abnormalities and poor growth (IOM 2002). ALA is the "parent" of the n-3 series and can be elongated and desaturated to longer chain, more highly unsaturated metabolites, beginning with eicosapentaenoic acid (EPA), which is in turn the precursor for series-3 eicosanoids and series-5 leukotrienes (IOM 2002). This metabolic chain is the basis for ALA's essentiality; it has no known specific function other than serving as a precursor for synthesis of EPA and docosahexaenoic acid (DHA).

Despite its high content of polyunsaturated fatty acids, flaxseed oil is quite stable. Malcolmson et al. (2000) stored samples of milled flaxseed at 23°C for 128 days and found no significant increase in peroxides, dienals, hexanal, or conjugated double bonds; increases in total volatiles were less than those reported in stored vegetable oils. A trained sensory panel detected no differences in the odor properties of fresh v. stored samples, nor could flavor differences be detected in bread made from fresh v. stored samples. Similarly, Przybylski and Daun (2001) reported that samples of milled flax that had been stored in warehouse conditions for up to 20 months suffered only very low levels of oxidative deterioration. Additionally, experiments on the effects of food processing and cooking on the lipid content of spaghetti containing ground flaxseed found that the ALA was stable through these processes (Manthey et al. 2002).

2.7.4. Protein

Protein content is typically estimated in flaxseed and other foods by measuring nitrogen content. For most food sources, a nitrogen conversion factor of 6.25 is used to estimate crude protein content. This value was based on early studies showing approximately 16% nitrogen in proteins from animal sources. A slightly lower conversion factor of 5.41 was estimated for flax meal based on amino acid analyses (Tkachuk 1969).

The protein content of flaxseed is about 10-31%, varying by cultivar, environmental conditions, and growing region, according to Hall et al. (2006), who also reported that a 1990 analysis of the protein content of eleven flaxseed cultivars from the U.S. (North Dakota) found a range of 26.9% to 31.6%, while a 1993 analysis of 11 Canadian cultivars found a range of 18.4% to 20.2%. These latter data were calculated from a report by Oomah and Mazza (1993), who reported the protein content of flaxseed on an oil-free and moisture-free basis, as shown in Table 10.

Table 10. Protein Content of Flaxseed Cultivars.

Cultivar	Protein (%; oil- and moisture-free basis)		
	1987	1988	1999
AC Linora	NA[1]	34.7	36.7
Andro	32.8	34.6	36.4
Dufferin	31.4	34.1	NA[1]
Flanders	32.5	34.6	36.0
Linott	32.5	34.9	36.5
McGregor	31.3	33.8	34.7
Noralta	31.4	33.9	35.8
NorLin	32.0	34.0	35.9
NorMan	32.5	34.6	36.2
Somme	32.4	34.4	36.3
Vimy	31.3	34.1	36.2

[1]NA= data not available
Source: Oomah and Mazza (1993)

Daun et al. (2003) reported that analyses of Canadian flaxseed over a 5-year span showed it to contain 23% crude protein with individual farm samples ranging from 17.4 to 29.2% and export samples ranging from 20.9-25.1%. Protein content is inversely related to oil content (correlation coefficient ≈ -0.5) based on data collected during Canadian Grain Commission harvest surveys from 1998 to 2002 (Daun et al. 2003).

Hall et al. (2006) reported that the high-molecular weight globulin fraction constitutes about 80% of total protein and the low-molecular weight fraction, mostly albumins, about 20%; similar proportions were estimated by Sammour (1999). Oomah and Mazza (1993), on the other hand, estimated the high-molecular weight fraction at 58% and the low-molecular weight fraction at 42%. These authors also reported that proteins in the high-molecular weight fraction have molecular weights in the range of 294 kilodaltons (kDa) while those in the low-molecular weight fraction are in the range of about 15-17 kDa. They also noted that flaxseed proteins are

only poorly soluble in water, being only 20-24% soluble between pH 2 and 6. Most of this solubility is provided by the low-molecular weight fraction.

2.7.4.1. Amino Acids

Based on their analysis of the amino-acid composition of three flaxseed cultivars (shown in Table 11), Oomah and Mazza (1993) concluded that glutamine is the most prevalent amino acid, followed by arginine and aspartic acid. The high-molecular weight fraction is rich in aspartic and glutamic acids, while the low-molecular weight fraction is characterized by large amounts of lysine, cysteine, glutamic acid, and glycine.

Table 11. Amino Acid Content of Flaxseed Cultivars.

Amino Acid as Percent of Protein	Cultivar		
	Norlin	Foster	Omega
Alanine	4.4	4.7	4.5
Arginine	9.2	10.0	9.4
Aspartic acid	9.3	10.0	9.7
Cysteine	1.1	1.8	1.1
Serine	4.5	4.7	4.6
Glutamine	19.6	20.0	19.7
Glycine	5.8	5.9	5.8
Histidine	2.2	2.1	2.3
Leucine	5.8	6.0	5.9
Isoleucine	4.0	4.1	4.0
Lysine	4.0	4.0	3.9
Methionine	1.5	1.4	1.4
Phenylalanine	4.6	4.8	4.7
Proline	3.5	3.8	3.5
Threonine	3.6	3.8	3.7
Tyrosine	2.3	2.4	2.3
Valine	4.6	5.1	4.7
Source: Oomah and Mazza (1993)			

The USDA nutrient database for standard reference (USDA/ARS 2007) also includes estimates of the amino acid content of flaxseed, reported as a percentage of the entire seed, as shown in Table 12.

Table 12. Amino Acid Content of Flaxseed.

Amino Acid	Content (g/100 g flaxseed)
Tryptophan	0.297
Threonine	0.766
Isoleucine	0.896
Leucine	1.235
Lysine	0.862
Methionine	0.370
Cystine	0.340
Phenylalanine	0.957
Tyrosine	0.493
Valine	1.072
Arginine	1.925
Histidine	0.472
Alanine	0.925
Aspartic acid	2.046
Glutamine	4.039
Glycine	1.248
Proline	0.806
Serine	0.970
Hydroxyproline	0.175
Source: USDA/ARS (2007)	

Hall et al. (2006) concluded that there is less variability in the amino acid content of flaxseed protein than the degree of protein variability across cultivars and growing regions.

2.7.5. Fiber

As noted earlier, the US nutrient database for standard reference (USDA/ARS 2007) reports that flaxseed contains 27.3 g total dietary fiber per 100 g flaxseed while the Canadian data (Health Canada 1999a) show 17 g fiber/100 g flaxseed. This difference may be due to the use of different cultivars, differences in methodologies (particularly extraction methods), or simple random variability. Over half of the dietary fiber found in flaxseeds is insoluble cellulose (Hodgins and Patterson 1994); Dahl et al. (2005) reported the proportion as 56%. The soluble fiber[†] in flaxseed is in the form of mucilage, also referred to as polysaccharide gum or

[†] As discussed in Section 3.3.1 on the definition of fiber, an Institute of Medicine Panel on the Definition of Dietary Fiber recommended phasing out use of the terms "soluble" and "insouble" to classify fibers (IOM 2001).

hydrocolloidal gum. (The presence of such mucilaginous material is uncommon among oilseeds [Mazza and Oomah 1995] although it is found in other sources such as mustard seed.) Flaxseed mucilage is present entirely in the seed coat, being on the epiderm of the seed coat (Bhatty 1993). This mucilage, which is a mixture of neutral and acidic polysaccharides varying in their galacturonic acid content, is responsible for the high water-hydration capacity of flaxseed products.

The mucilage content of processed flaxseed from 3 cultivars, Andro, Linott, and Norlin, was estimated at 5.0, 5.0, and 5.3%, respectively (Bhatty 1993). An early study (Anderson and Lowe 1947) identified the composition of flaxseed mucilage as shown below in Table 13. The researchers noted that there was no positive evidence of any sugar other than the 3 listed in the table and argued that the physical properties of the mucilage (resembling those of a starch) suggested a branched chain structure.

Table 13. Composition of Flaxseed Mucilage

Component	Percent[1]
Xylose	31.36
Galactouronic acid	30.46
Galactose	23.88
Rhamnose	14.30

[1] Presented on an ash-free basis
Source: Anderson and Lowe (1947)

More recently, using improved extraction and analytical techniques, Oomah et al. (1995a) analyzed the mucilage content of 109 samples of several varieties of flaxseed from 12 geographical areas. Total water-soluble carbohydrate contents ranged from 3.6% to 8% of the flaxseed with a mean of 6.2%. In contradistinction to Anderson and Lowe (1947), Oomah et al. (1995a) found that glucose is the major monosaccharide in flaxseed. They attributed this difference to their use of a phosphate buffer (pH 7.0) rather than pure water for the extraction. The polysaccharide content of flaxseed as determined by Oomah et al. (1995a) is shown in Table 14.

Table 14. Ranges of Composition of Flaxseed Mucilage.

Component	Percent		
	Minimum	Mean	Maximum
Glucose	21.3	28.9	40.0
Xylose	10.9	23.2	32.0
Galactose	12.7	19.1	26.9
Rhamnose	8.2	14.2	23.8
Arabinose	3.2	10.6	15.4
Fucose	1.9	3.5	9.1
Source: Oomah et al. (1995a)			

Aside from the presence of glucose, the ordering of the sugars by Oomah et al. (1995a) is the same as that in the analyses of Anderson and Lowe (1947)—xylose, galactose, rhamnose—although the more recent analyses also identified two sugars, arabinose and fucose, that were not found in the earlier work. Oomah et al. (1995a) also confirmed the branched chain structure hypothesized in the earlier research. Mazza and Oomah (1995) reported that the neutral polysaccharide is primarily composed of a backbone of a (1→4)-linked β-D-xylopyranose chain which is 0-2 substituted with side chains of (1→5) or (1→3)-linked arabinose. The principal polysaccharide structure in the acidic fraction is a backbone of (1→2)-linked L-rhamnose units with some branching at the 0-3 positions, and with D-galacturonic acid present as (1→4)-linked residues located either in the main chain or as part of side chains. Other chemical structures exist in smaller concentrations.

Oomah et al. (1995a) also commented on the high degree of variation in the relative concentrations of the different sugars, which did not appear to be closely related to cultivar or growing area. Although they did not report the data, they also analyzed their samples for total protein and lipid content and found no correlation between either of these parameters and either the total polysaccharide content or the sugars distribution.

The functionality of flaxseed mucilage was explored by Mazza and Biliaderis (1989) and compared with that of locust bean, guar gum, and gum arabic. They found that extraction with water at 25°C yielded 3.5 to 5.5% mucilage, similar to the findings of Bhatty (1993) and Oomah et al. (1995a), reported above. The use of boiling water increased yields to nearly 9% but resulted in a darker finished product, and so later extractions were performed by beginning with boiling water (100°C) and allowing it to cool to room temperature (25°C). (The darkness of the gum extracted at high temperatures may have been due to protein; Mazza and Oomah (1995) reported that hot-water extraction produces a gum with a higher protein content than does cold-water extraction.) Mazza and Biliaderis' (1989) results are summarized in Table 15, below.

Table 15. Flaxseed Mucilage Extraction by Time and Temperature.

Extraction Time (Hours)	Mucilage Concentration (% Flaxseed)		
	25°C	100-25°C	100°C
0.5	3.6	6.5	8.4
1.0	4.4	7.2	8.5
2.0	4.8	7.6	8.6
4.0	5.2	7.8	8.9
6.0	5.2	7.9	8.9
8.0	5.3	8.2	8.9
Source: Mazza and Biliaderis (1989)			

Mazza and Biliaderis (1989) also explored the composition of the mucilage extracted at the various temperatures in 2 hours and compared it with guar gum and locus bean gum, as shown in Table 16.

Table 16. Composition of Flaxseed Mucilage and Other Gums.

Constituent	Unit	Flaxseed Extraction Temperature			Guar Gum	Locust Bean Gum
		25°C	100-25°C	100°C		
Water	%	7.5	4.5	5.1	8.8	8.9
Ash	%	11.8	11.4	11.4	0.7	0.9
Protein	%	4.6	12.8	14.5	4.5	7.4
Lipid	%	0.4	0.2	0.5	0.7	1.2
Carbohydrate	%	83.3	75.5	73.6	94.2	90.5
Potassium	%	2.9	4.1	4.2	0.2	0.4
Calcium	%	1.3	0.9	0.8	0.1	0.1
Magnesium	%	0.5	0.4	0.3	0.1	0.1
Phosphorus	%	0.1	0.2	0.2	0.1	0.1
Iron	ppm	83	66	66	49	29
Zinc	ppm	59	46	42	14	15
Manganese	ppm	24	15	14	2	4
Copper	ppm	13	22	14	2	5
Source: Mazza and Biliaderis (1989)						

The most obvious difference between flaxseed mucilage and both guar and locust bean gum is the higher ash and mineral content. However, the guar and locust bean gums were commercial products and it is not known what sort of processing they might have undergone.

The solubility of a 0.5% solution of flaxseed mucilage was between 70 and 90%, differing little from the solubilities of gum arabic, guar gum, and locust bean gum. The viscosity of flaxseed mucilage at low shear rates was between that of gum arabic and locust bean or guar gums, although Mazza and Oomah (1995) observed that the extraction conditions can influence the viscosity of the gum. Mazza and Biliaderis (1989) found that the order of increasing viscosity among carbohydrate hydrocolloids was potato starch < gum arabic < flaxseed mucilage < locust bean < guar < xanthan. At high shear rates these differences were minimized. The viscosity of flaxseed mucilage was higher at neutral pH (6-8) than in either acidic or alkaline solutions. Finally, the water sorption characteristics of flaxseed mucilage were similar to those of other plant gums and were dependent on temperature.

Mazza and Oomah (1995) reported that the water-binding capacity of the gum extracted from 3 cultivars of flaxseed ranged between 1600 and 3000 g H_2O/100 g solids, similar to that of guar gum.

2.7.6. Micronutrients

2.7.6.1. Minerals

The mineral content of flaxseed, based on analyses of 7 samples, is included in the USDA/ARS (2007) nutrient database. The Health Canada (1999a) tables also include the flaxseed content of 5 minerals. These data are displayed in Tables 17 and 18, below.

Table 17. Mineral Content of Flaxseed (USDA/ARS).

Mineral	Unit	Content (per 100 g)
Calcium	mg	255
Iron	mg	5.7
Magnesium	mg	392
Phosphorus	mg	642
Potassium	mg	813
Sodium	mg	30
Zinc	mg	4.3
Copper	mg	1.2
Manganese	mg	2.5
Selenium	µg	25.4
Source: USDA/ARS (2007)		

Table 18. Mineral Content of Flaxseed (Health Canada).

Mineral	Unit	Content (per 100 g)
Calcium	mg	268
Iron	mg	11.1
Potassium	mg	1301
Sodium	mg	66
Zinc	mg	7.7
Source: Health Canada (1999a)		

Cadmium has been identified in flaxseed, as in many other plants, and levels in flaxseed are dependent on cadmium concentrations in soil (Grant and Bailey 1997; Grant et al. 2000) and the variety of seed (Chen et al. 1999; Hocking and McLaughlin 2000; Hammond et al. 2000; Chaney et al. 2001; Oomah et al. 2007). Cadmium levels identified in a large selection of varieties from the USDA collection grown on soil with high cadmium levels ranged from 0.2 to 3.6 mg/kg (Hammond et al. 2000). In studies of Canadian flaxseed, the range was 0.2 to 0.6 mg/kg (Daun and Przybylski 2000a) and the highest level noted by the Canadian Grain Commission was one sample with a level above 3 mg/kg. It has been reported that the risk to health of cadmium ingestion at the levels reported in flaxseed is considerably less than that of other cereals such as wheat or rice (Butler and Conn 1964).

2.7.6.2. Vitamins

The vitamin content of flaxseed as reported in the USDA/ARS (2007) database, based on analyses of from 1 to 6 samples, is shown in Table 19, below. The vitamin contents of flaxseed listed in the Health Canada (1999a) tables are shown in Table 20.

Table 19. Vitamin Content of Flaxseed (USDA/ARS).

Vitamin	Unit	Content (per 100 g)
Vitamin C	mg	0.6
Thiamin	mg	1.64
Riboflavin	mg	0.16
Niacin	mg	3.08
Pantothenic acid	mg	0.98
Vitamin B-6	mg	0.47
Folate	µg	87
Choline	mg	78.7
Betaine	mg	3.1
Vitamin B-12	µg	0
Vitamin A	IU	0
Vitamin E	mg	0.31
γ-tocopherol	mg	19.95
δ-tocopherol	mg	0.35
Vitamin K	µg	4.3
Source: USDA/ARS (2007)		

Table 20. Vitamin Content of Flaxseed (Health Canada).

Mineral[1]	Unit	Content (per 125 ml or 73 g)	Content (per 100 g)
Vitamin C	mg	1	1.36
Thiamin	mg	0.12	0.16
Riboflavin	mg	0.12	0.16
Niacin	NE*	3.4	4.7
Vitamin B-6	mg	0.55	0.75
Folate	µg	218	299

[1]values are given for the weight of the edible portion as described (i.e., flaxseeds)

*Niacin Equivalent (= 1 mg niacin or 60 mg tryptophan)

Source: Health Canada (1999a)

2.7.7. Lignans

Phenolic compounds are widely distributed in plants; they occur in many forms, but in oilseed products they occur primarily as the hydroxylated derivates of benzoic and cinnamic acids, coumarins, flavonoid compounds, and lignins (Oomah et al. 1995b). The phenolic acid content of flaxseed is addressed below; this section focuses on lignans.

Lignans are a class of naturally occurring compounds characterized by the bonding of two aryl propanoids (cinnamic acid residues) at the 2-position. Lignans are usually found as phenylpropanoid dimers in which the phenylpropanoid units are linked tail to tail and thus have a 2,3 dibenzylbutane skeleton, but higher oligomers can also exist.

The primary lignan in flaxseed is 2,3-bis(3-methoxy-4-hydroxybenzyl)butane-1,4-diol, more often referred to by the trivial name secoisolariciresinol (SECO; Lampe et al. 2006). SECO (CAS number 29388-59-8) has the molecular formula $C_{20}H_{26}O_6$ and a molecular weight of 362.4. In flaxseed, SECO is usually bonded to two glucose units to form a diglucoside, secoisolariciresinol diglucoside (SDG). The full chemical name is β-D-glucopyranoside,2,3-bis[(4-hydroxy-3-methoxyphenyl0methyl]-1,4-butanediyl-bis-[R-(R*,R*)]. It is a member of the oxydiarylbutan class of lignans (Meagher et al. 1999). SDG has CAS number 158932-33-3; its molecular formula is $C_{32}H_{46}O_{16}$ and its molecular weight is 686.7. The chemical structure of SDG is shown below in Figure 3. SDG does not exist in flaxseed in free form, but rather is part of a soluble ester-linked complex that also contains 3-hydroxyl-3-methyl-glutaric acid and a number of cinnamic acid glucosides (Muir 2006).

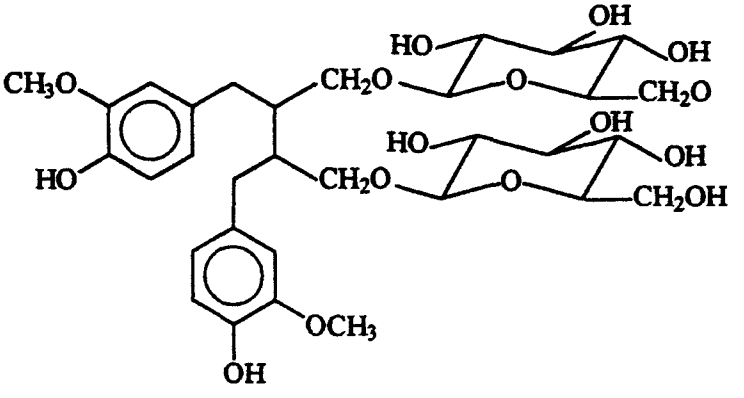

Figure 3. Secoisolariciresinol Diglucoside (SDG; from Rickard et al. 2000).

SDG was first isolated in 1956 by Bakke and Klosterman (Muir 2006), but no biological activity was recognized at that time; it was only after the 1980 discovery of the mammalian lignans enterolactone and enterodiol that SDG was recognized (in 1982) as the principal precursor. (The kinetics of SDG are discussed in detail in a later section of this monograph.)

The stability of SDG derived from flaxseed under conditions of baking and during storage was tested by Hyvarinen et al. (2006). SDG in rye breads, graham buns, and muffins was analyzed by HPLC before and after baking and no change was found; similarly, SDG in buns containing fat-free flaxseed meal was unaffected by storage for 1 week at room temperature or for up to 2 months at -25°C.

A second lignan in flaxseed that can also serve as a precursor to the mammalian lignans is matairesinol, [3R-*trans*]-dihydro-3,4-bis[(4-hydroxy-3-methoxyphenyl)-methyl]-2(3*H*)-furanone (Mazur et al. 1996), a member of the diarylbutyrolactone class of lignans (Meagher et al. 1999). The molecular structure of this lignan is shown in Figure 4.

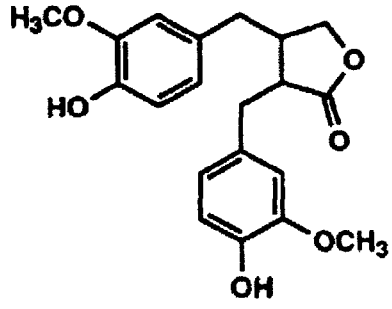

Figure 4. Matairesinol (from Mazur et al. 1996).

Mazur et al. (1996) analyzed a number of food products for their lignan content. Flaxseed had by far the highest concentration of SDG of any food tested. The SECO content (i.e., the residue of SDG after removal of the 2 glucosides) of flaxseed was 3699.0 μg/g, more than 2 orders of magnitude higher than the next highest food tested, lapacho tea, containing 26.7 μg/g SECO, followed by Finnish country rye bread and sunflower seeds with 11.53 μg/g and 6.095 μg/g, respectively. Only 0.707 μg SECO/g was found in American 9-grain bread. (Since SECO constitutes 52.8% of the SDG molecule, the SDG content of flaxseed is 1.89 x SECO, or 7009 μg/g flaxseed.)

Flaxseed was also an abundant source of the lignan matairesinol (Mazur et al. 1996), with 10.87 μg/g, although lapacho tea was an even better source with 16.70 μg/g. No other food reported in this study contained as much as 1 μg/g of matairesinol.

Two other lignans identified in flaxseed are not known to be capable of conversion to enterodiol or enterolactone, although they may be precursors of other mammalian lignans (Sicilia et al. 2003).. These are pinoresinol ([1*S*-(1α,3α,4β,6α)]-4,4'-(tetrahydro-1*H*,3*H*-furo(3,4-)furan-1,4-diyl)bis(2-methoxyphenol), a furofuran lignan, and isolariciresinol ([1S-(1α,2β,3α)]-1,2,3,4-tetrahydro-7-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-6-methoxy-2,3-naphthalenedimethanol), a tetrahydronaphthalene lignan (Meagher et al. 1999). Prior to their identification in flaxseed, isolariciresinol had been detected only in human urine, and pinoresinol—as implied by its name—had been found primarily in coniferous trees (Meagher et al. 1999).

In a more recent analysis using improved extraction methods, Smeds et al. (2007) assessed the lignan contents of a variety of cereals, oilseeds, and nuts. A number of lignans isolated from flaxseed had not previously been reported. The lignan content of flaxseed as determined by Smeds et al. (2007) is shown below in Table 21.

Table 21. Lignan Composition of Flaxseed.

Lignan	Concentration (µg/100 g)
Secoisolariciresinol	165759
Secoisolariciresinol-sesquilignan	6326
Cyclolariciresinol	3726
Lariciresinol	1780
Hydroxysecoisolariciresinol	958
Pinoresinol	871
Metairesinol	529
Lariciresinol-sesquilignan	86
Syringaresinol	48
Hydroxymetairesinol	35
Nortrachelogenin	33
Oxometairesinol	10
Dimethylmetairesinol	1
Source: Smeds et al. (2007)	

As noted previously, the SDG content of flaxseed is 1.89 x SECO and thus can be estimated as 3133 µg SDG/g or 3.13 mg/g flaxseed according to the analysis by Smeds et al. (2007). This is approximately half the 7.01 µg/g concentration found by Mazur et al. (1996). Yet another analysis of the SDG content of flaxseed was conducted by Johnsson et al. (2000) using HPLC; this analysis found a range of 6.1 mg SDG/g to 13.3 mg SDG/g. It is clear from these disparate findings that analytical results are highly dependent not only on the SDG content of the different cultivars and growing conditions of flaxseed, but also the extraction and analytical methods employed.

Few lignans in flaxseed exist in aglyconic form (Smeds et al. 2007). As was noted above, secoisolariciresinol always occurs as SDG, the diglucoside form. Only 6% of pinoresinol is present as an aglycone, as is 7% of lariciresinol and 16% of cyclolariciresinol. The only exceptions are metairesinol and hydroxymetairesinol; 41% and 58%, respectively, exist as aglycones. (Smeds et al. (2007) noted that this is not the case with many cereals such as rye, wheat, barley, and buckwheat, in which most of the lignans are present as aglycones.)

Smeds et al. (2007) concluded that flaxseed and sesame seed are by far the most lignan-rich species used as foodstuffs. Although flaxseed has long been regarded as the best single source of lignans, inclusion of the lipophilic sesamin-type lignans (which had previously been under-estimated) moves sesame seed into the lead. The highest lignan content among cereal grains is found in rye and wheat.

2.7.8. Phenolic Acids

Radtke et al. (1998) suggested that the antioxidant and anticarcinogenic properties of phenolic acids could be part of the cause of the inverse association between fruit and vegetable intake and the incidence of coronary heart disease and cancer. The estimated daily intake of phenolic acid in a Bavarian subpopulation of the German National Food Consumption Survey was 222 mg/day, with caffeic acid accounting for 206 mg/day of this total (Radtke et al. 1998).

Oomah et al. (1995b) analyzed the phenolic acid content of 96 samples of flaxseed from 8 cultivars grown at 4 locations in western Canada for 3 years. The results by cultivar are shown below in Table 22.

Table 22. Phenolic Acid Concentrations in Flaxseed Cultivars.

Cultivar	Phenolic Acids (mg/g)		
	Total	Esterified	Etherified
AC Emerson	9.92	4.79	5.13
AC Linora	10.34	5.38	4.97
Flanders	8.46	5.12	3.34
Linola 947	7.89	5.19	2.67
McGregor	8.13	5.42	2.72
NorLin	9.48	5.21	4.27
Somme	8.85	5.42	3.44
Vimy	9.52	5.30	4.22
Source: Oomah et al. (1995b)			

Growing location had no consistent effect on the phenolic acid content of flaxseed, but there were significant differences across cultivars, particularly in etherified phenolic acids, predominantly ferulic acid. The largest source of variability was seasonal effects. However, the phenolic acid content of flaxseed was independent of the protein and oil content (Oomah et al. 1995b).

2.7.9. Cyanogenic Glycosides

Like a large number of other edible plants, flaxseed contains 4 glycosides (linustatin, neolinustatin, linamarin, and lautostralin) that release hydrogen cyanide on hydrolysis (Hall et al.

2006). These glycosides are synthesized from amino acids, including leucine, isoleucine, and valine (Barthet and Daun 2000). The major cyanogenic glycosides in fully mature seeds are both diglucosides: linustatin, β-gentiobioside of acetone cyanohydrin, and neolinustatin, methylethyl ketone cyanohydrin (Oomah et al. 1992; Daun 2008a). Neolinustatin differs from linustatin by having an ethyl group rather than a methyl group adjacent to the cyano group. The monoglycosides (linamarin and lautostralin) appear in more immature seeds or as artifacts in methodologies which do not inactivate the seed enzymes linustatinase and linamarinase (Daun 2008a). Hydrolysis of the cyanogenic glycosides in the presence of the 2 glucoside enzymes linustatinase and linamarinase forms a hydroxypropanenitrile which decomposes in aqueous solution to hydrogen cyanide. Daun (2008a,b) provided a schematic of the enzymatic hydrolysis of linustatin to hydrogen cyanide and acetone, shown below in Figure 5.

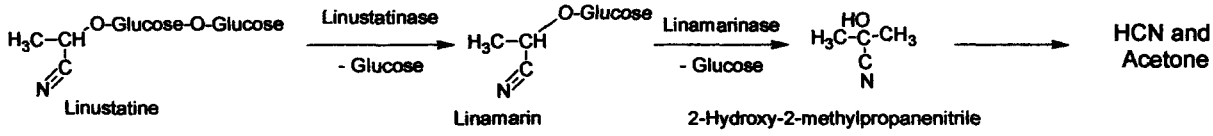

Figure 5. Enzymatic Hydrolyis of Linustatin (from Daun 2008a,b).

According to research by Frehner et al. (1990), the distribution of the cyanogenic glycosides varies with the age of the flaxseed at time of harvest; while only the monoglycosides are present at anthesis, only the diglucosides are present at full maturity. Both types of glycosides are present at intermediate stages of development.

The structural formulas of these glycosides are shown in Figures 6-9 below (from Hall et al. 2006).

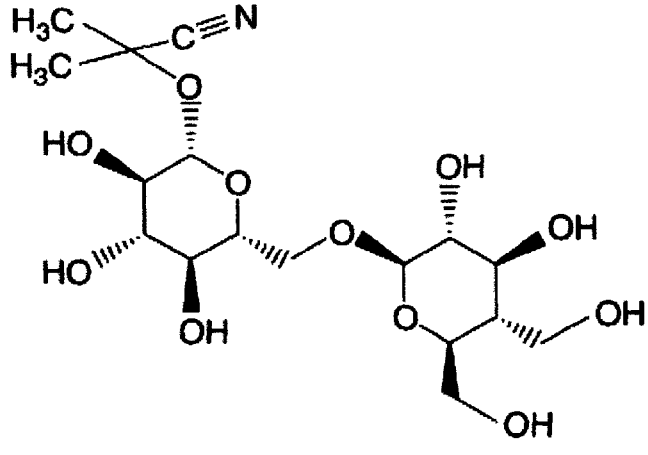

Figure 6. Linustatin.

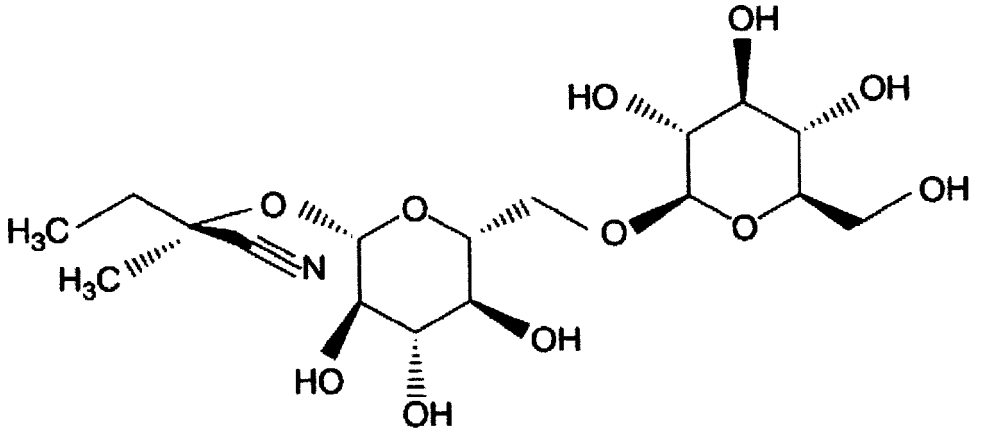

Figure 7. Neolinustatin.

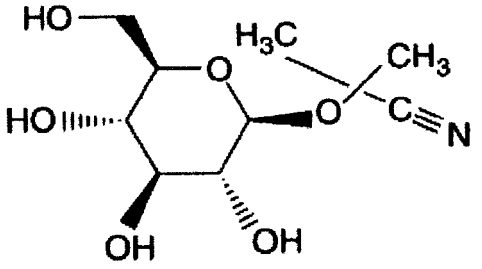

Figure 8. Linamarin.

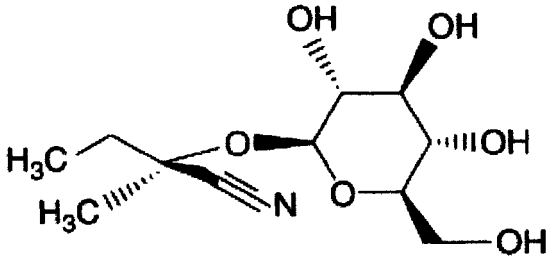

Figure 9. Lautostralin.

The cyanogenic glycoside content of flax differs depending on location in the plant and stage of development (Hall et al. 2006). Oomah et al. (1992) analyzed the cyanogenic glycosides present in 10 Canadian cultivars and found linustatin and neolinustatin to be the most abundant compounds with linamarin present as low levels (Table 23); the detection of linamarin may be an artifact of the methodology (Daun 2008, personal communication).

Table 23. Cyanogenic Glycoside Content of Flaxseed Cultivars.

Cultivar	Cyanogenic Glycoside Content (mg/g seed)		
	Linamarin	Linustatin	Neolinustatin
AC Linora	0.20	2.69	1.22
Andro	0.17	3.42	2.03
Flanders	0.14	2.82	1.47
Linott	0.22	2.13	1.61
McGregor	0.26	3.52	0.91
Noralta	0.20	2.71	1.63
Norlin	ND	2.95	2.01
NorMan	ND	2.31	1.35
Somme	0.28	3.22	1.49
Vimy	0.32	2.62	1.15
Source: Oomah et al. (1992)			

The total cyanogenic glycoside content of the cultivars tested by Oomah et al. (1992) ranged from 3.66 mg/g (in NorMan) to 5.62 mg/g (in Andro); the overall mean was 4.51 mg/g.

In a biotechnology consultation filed with FDA for sulfonylurea-tolerant genetically modified flaxseed (FDA 1998), the developer of the modified strain stated that the typical range of cyanogenic glycoside contents for Canadian cultivars is 6.0 to 9.2 mg/g seed; the modified strain contained a concentration of 7.7 mg/g.

Bhatty (1993) found strong seasonal effects on the total cyanogenic glycoside content of 8 flaxseed cultivars, while Oomah et al. (1992) determined that the concentrations of all 3 cyanogenic glycosides were dependent on cultivar, location, and year of production, with cultivar being the most important factor. Studies by Daun and Przybylski (2000b) showed that the concentrations of cyanogenic glycoside in seeds vary signficantly with both cultivar and environment (expressed as oil content), as shown in Table 24.

Table 24. Effect of Variety and Oil Content On Cyanoglycosides in Canadian Flax Cultivars.

Variety	Oil Content	Linustatin (mMol/kg)	Neolinustatin (mMol/kg)	Total (mMol/kg)	As HCN (mg/kg)
NorLin	High	4.55	5.70	10.25	297
	Medium	4.55	5.86	10.41	302
	Low	4.55	5.56	10.10	293
Vimy	High	4.94	4.35	9.29	269
	Medium	4.89	4.42	9.31	270
	Low	4.35	4.11	8.47	246
Flanders	High	5.16	4.18	9.34	271
	Medium	4.84	4.73	9.57	278
	Low	4.65	4.02	8.66	251
Somme	High	6.36	5.27	11.63	337
	Medium	5.50	4.47	9.97	289
	Low	5.13	4.70	9.84	285
All Varieties	Mean	4.96	4.78	9.74	282
	Minimum	4.35	4.02	8.47	246
	Maximum	6.36	5.86	11.63	337
	Std. Dev.	0.55	0.65	0.84	24.5
	CV[1]	11.07	13.61	8.66	8.7
Statistical Significance	p(Oil)	0.027	0.38	0.117	0.117
	p(Variety)	0.007	0.003	0.022	0.022

1. Coefficient of variation

Source: Daun and Przybylski (2000b)

A number of studies have examined the effect of processing on the cyanogenic glycoside content of flaxseed and its products (Wanasundara et al. 1993; Shahidi et al. 1997; Oohma and Mazza 1998; Feng et al. 2003; Yang et al. 2004). Overall, there is no evidence demonstrating that it is possible to remove the cyanogenic glycosides in intact flaxseed by physical treatment (e.g., heat) without severely affecting the quality of the seed. Treatments that appear to reduce the amount of cyanogenic glycosides in intact seeds do so by inactivation of the glycosidase enzymes.

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2.7.9.1. Hydrogen Cyanide

When flaxseed is crushed in water, hydrogen cyanide is released from these cyanogenic glycosides by the action of the enzyme β-glycosidase, which is present in the flaxseed itself (Chadha et al. 1995). This released cyanide is then washed away.

Cyanide released from flax is estimated either by direct measurement of the cyanide released by hydrolysis of the cyanogenic glycosides or by measuring the amount of cyanogenic glycosides present and then calculating the maximum amount of cyanide potentially released (Daun 2008a). Measurement of cyanogenic glycosides by HPLC (Oomah et al. 1992) or GC (Bacala and Barthet 2007) gives results comparable to those obtained by direct estimation based on measuring cyanide (Kobaisy et al. 1996) produced on hydrolysis if intact enzyme systems are present (Daun 2008b). However, if either of the seed enzymes is inactivated, even if both are present, the direct method of measurement of cyanide produced by hydrolysis yields low or negative results (Daun 2008b). Some hydrogen cyanide is lost to the atmosphere in most methodologies; additionally, most methods rely on complete hydrolysis of the glycosides by endogenous seed enzymes (Daun 2008a).

Table 25 on the following page provides a summary of the concentrations of cyanogenic glycosides and of hydrogen cyanide found in flaxseeds from different sources using a variety of analytical techniques. It is also to be noted that immature seeds have much higher levels (712-1243 ppm) of hydrogen cyanide than do fully mature seeds (78.3-570 ppm).

Using colorimetric methods, Bhatty (1993) measured hydrogen cyanide concentrations of 8 freshly ground flaxseed cultivars grown in 4 locations in 1987, and of 7 freshly ground flaxseed cultivars grown in 1987 and 1989. Based on these studies, Bhatty (1993) concluded that cultivar, growing location, and season all affect the cyanide concentration in flaxseeds, with the yearly variation being the largest contributor to the variance. The overall mean concentration of hydrogen cyanide in flaxseed from both studies was 8.8 μg/100 g flaxseed, or 0.09 μg/g, with a range of 0.06 to 0.12 μg/g. The levels reported by Bhatty (1993) are approximately 1000x lower than those reported by other authors (see Table 25), and it is possible that an error was made in the article and that the units should have been mg rather than μg.

Table 25. Summary of Cyanogenic Glycoside and Hydrogen Cyanide Contents of Flaxseed.

Source	Method	g/100g seed				Total HCN Potential (mg/kg)	Measured HCN (mg/kg)
		Linustatine	Neolinustatine	Linamarin	Lautostralin		
Canada (Cunnane et al. 1993)	HPLC	0.0003	0.0004	0.0000	0.0000	0.40	
Canada (Oomah et al. 1992)	HPLC/UV	0.168 – 0.380	0.072 – 0.238	0 – 0.403	0.0000	157 – 448	
Canada (Kobaisy et al. 1996)	HPLC						270-570
Unknown (Schilcher et al. 1986)	HPLC	0.218 – 0.538	0.083 – 0.454	0.0000	0.0000	197 – 647	
Seed (Chadha et al. 1995)	Ion chromatography after autolysis						124-196
Canada (Haque and Bradbury 2002)	Acid hydrolysis						360
	Picrate						360
Australia (Haque. and Bradbury 2002)	Acid hydrolysis						140-300
	Picrate						140-350
Meal (Canada) (Wanasundara et al. 1993)	HPLC	0.265	0.114	0.0000	0.0000	249	
Immuature seed of unknown origin (Niedzwiedz-Siegien 1998)	TLC	0.200 – 0.320	0.106 – 0.185	0.333 – 0.605	0.141 – 0.240	712 – 1243	
Seed (Zöllner and Giebelmann 2007)							210-540
Cake (Zöllner and Giebelmann 2007)							500
Sprouts (Zöllner and Giebelmann 2007)							9100
Food grade seed (Barthet 2006)	GC	0.132-0.246	0.153-0.261			209-309	
Various Canada (Daun and Przybylski 2000b)	HPLC	0.178-260	0.170-0.248			245-337	
Seed, survey of 104 cultivars, world wide (Oomah et al. 1995c)		0.073-0.395	0-0.315			78.3-353.8	

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2.7.10. Linatine

Linatine (1[(N-γ-glutamyl)-amino]-D-proline) is a pro-antivitamin B_6 antagonist and has been detected at levels of about 100 mg/kg in flaxseed (Klosterman et al. 1967; Klosterman 1974). As shown in Figure below, linatine is hydrolyzed (presumably through a protease enzyme in the gut) to 1-amino-D-proline (DAP) which is the only vitamin B_6 antagonist resulting from natural feedstuff:

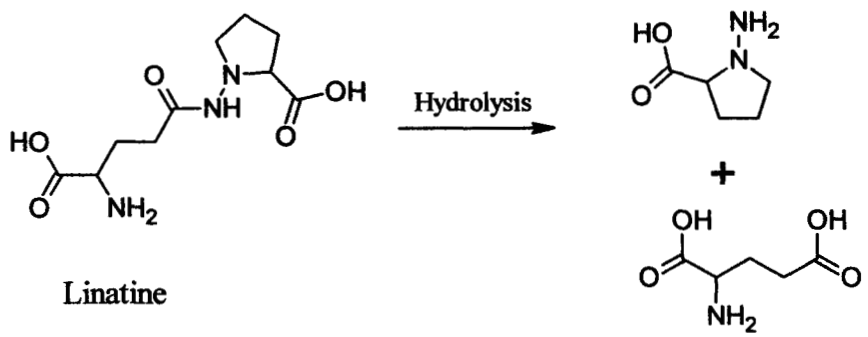

Figure 10. Linatine Hydrolysis (from Klosterman 1974)

Once DAP is released, it can form a stable derivative with pyridoxal or pyridoxial phosphate (vitamin B_6) resulting in a loss of available vitamin B_6 (Daun 2008c).

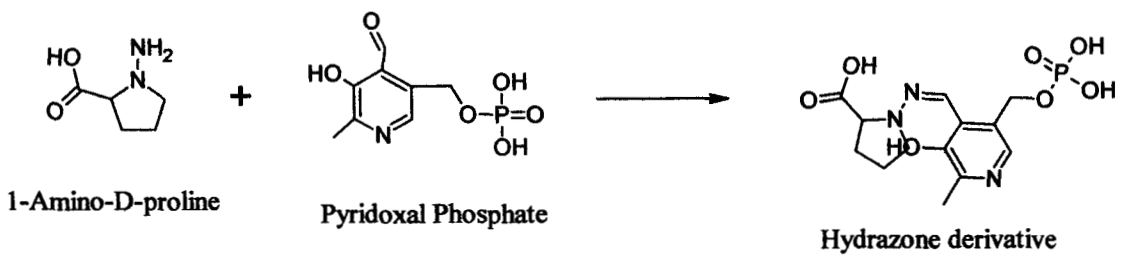

Figure 11. Formation of Hydrazone Derivative.

Addition of vitamin B_6 to the diet readily alleviates this problem. Vitamin B_6 deficiency through ingestion of linatine in flaxseed is not expected to be an issue at the intended use levels due to adequate exposure to vitamin B_6 in the diet.

2.8. Physical and Chemical Properties

A brief summary of the physical and chemical properties of flaxseed is provided in Table 26.

Table 26. Physical Characteristics of Whole and Milled Flaxseed.

Physical Standard	Whole	Milled
Appearance	Uniform brown or golden color	Uniform golden color with or without brown flecks
Odor	No off odors	No off odors
Texture	Smooth, free-flowing granulation	Free-flowing powder
Bulk density	c. 65 kg/hl	c. 48 kg/hl
Particle sizes	100% passes through 3.5/64" x 1/2" slotted hole or 4.5/64" round hole	Variable

2.9. Production Process

Flaxseed is produced under Good Manufacturing Practice (GMP) as described below.

2.9.1. Sorting and Cleaning

This process involves subjecting the flaxseed to a variety of separations that are based on differences in length, width and bulk density to remove dirt and other extraneous material. Typical cleaning equipment would include aspirators, scalpers, indents, graders, de-stoners, and gravity equipment.

In some cases electronic color sorting may be used to improve the aesthetics and quality of the food grade flaxseed product.

2.9.2. Milling; ranges of particle sizes

The milling of flaxseed is different from corn or cereal milling due to the high fat content. The object is to produce a granular, non-oily, free-flowing product. It is desirable to mill flaxseed with mills designed to micronize or cut the flaxseed as opposed to crushing. It is desirable to use a process that does not significantly damage the oil-rich cellular structure of the seed. Such grinding or milling processes produce a free-flowing granular product with particle sizes ranging from 200 to 1500 μm.

2.9.3. Additional Processing

Although flaxseed is considered a low risk product in terms of microorganisms and pathogens, some processors have developed processes to "pasteurize" or significantly reduce the microbial load prior to further processing.

Permitted anti-caking agents such as silica may be used to improve the flowability of milled product.

2.10. Specifications for Food-Grade Material

Specifications for food-grade whole and milled flaxseed are shown in Table 27, along with the results of analyses of 6 samples of product. All samples were within specification for all parameters, indicating that the production process is consistently in control.

Table 27. Food Grade Specifications

Parameter (whole and milled flax)	Unit	Specification	Analytical Results					
			#569	#570	#591	#640	#681	#731
Proximates								
Moisture (%)	%	NLT[1] 3, NMT[2] 10	6.83	7.09	6.89	6.98	6.58	7.28
Protein (%	%	NLT 15, NMT 30	18.3	19.5	18.2	20.5	21.2	18.4
Carbohydrate	%	NLT 25, NMT 38	33.6	34.3	34.9	29.0	29.4	33.1
Dietary fiber	%	NLT 20, NMT 37	23.2	23.9	24.7	24.7	22.5	23.0
Total fat	%	NLT 32, NMT 45	37.98	35.53	36.70	39.97	39.21	37.70
Free fatty acids	g/100 g fat	NMT 2.0	0.40	0.46	0.41	0.60	0.51	0.35
Peroxides	meq/kg fat	NMT 5.0	0.00	0.13	0.00	0.00	0.36	0.00
Heavy metals								
Lead	mg/kg	NMT 0.5	<0.325	<0.300	<0.246	<0.240	<0.249	<0.249
Cadmium	mg/kg	NMT 1.0	0.238	0.302	0.223	0.244	0.354	0.239
Mercury	mg/kg	NMT 0.1	0.010	<0.007	<0.005	0.015	<0.005	--
Arsenic	mg/kg	NMT 3.5	0.04	<0.04	).03	0.03	0.03	--
Microbial								
Aerobic plate count	cfu[3]/g	NMT 1,000,000	30,000	18,000	60,000	150,000	50.000	40,000
Coliforms	cfu/g	NMT 100	<10	<10	<10	<10	<10	<10
E. coli	cfu/g	NMT 10	<10	<10	<10	<10	<10	<10
Salmonella		negative in 25 g	Passed	Passed	Passed	Passed	Passed	Passed
Listeria		negative in 25 g	Passed	Passed	Passed	Passed	Passed	Passed
Staphylococcus aureus	cfu/g	NMT 10	<10	<10	<10	<10	<10	<10
Yeast	cfu/g	NMT 2000	160	360	<10	800	500	<10
Mold	cfu/g	NMT 3000	110	<10	180	1600	70	1100

1. NLT – not less than
2. NMT = not more than
3. cfu = colony forming units

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2.11. Contaminants

Four samples of flaxseed were analyzed for concentrations of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), and the oil from these samples was analyzed for dioxin-like compounds. No contaminants were present in concentrations exceeding acceptable limits, based on calculation of World Health Organization (WHO) Toxic Equivalent values.

2.11.1. PCBs

Congener Group	PCB Concentration (ppt)			
	753	754	755	756
Monochlorinated[1]	15.9	37.2	45.4	91.1
Dichlorinated[2]	157	38.2	230	595
Trichlorinated[3]	13.5	55.2	46.2	84.2
Tetrachlorinated[4]	8.6	9.2	ND	6.3
Pentachlorinated[5]	ND	5.5	ND	4.8
Hexachlorinated[6]	ND	ND	ND	ND
Heptachlorinated[7]	ND	ND	ND	ND
Octachlorinated[8]	ND	ND	ND	ND
Nonachlorinated[9]	ND	ND	ND	ND
Decachlorinated[10]	ND	ND	ND	ND
Total	195	145	322	781
WHO TEQ[12]	0.05	0.05	0.09	0.09

1. PCBs 1 and 3
2. PCBs 4, 6, 8, 10, and 15
3. PCBs 16, 18, 19, 28, 31, and 33
4. PCBs 40, 41, 44, 49, 52, 54, 60, 66, 70, 74, 77, and 81
5. PCBs 84, 85, 87, 95, 97, 99, 101, 104, 105, 110, 114, 118, 119, 123, and 126
6. PCBs 29, 135, 137, 138, 141, 149, 151, 153, 155, 156, 157, 158, 167, 168, and 169
7. PCBs 170, 171, 174, 177, 178, 180, 183, 187, 188, 189, 191, and 193
8. PCBs 199, 200, 201, 202, and 205
9. PCBs 206, 207, and 208
10. PCB 209
11. ND = not detected
12. World Health Organization Toxic Equivalent (calculated with non-detects set to the limit of detection)

2.11.2. PAHs

PAH	PAH Concentration (ppb)			
	753	754	755	756
Tetrain	1.6	ND (0.6)*	ND (1.3)	ND (1.4)
Naphthalene	9.2	8.1	11.5	18.5
Quinoline	1.6	ND (0.4)	3.2	4.3
2-Methylnaphthalene	5.3	4.4	5.8	9.2
1-Methylnaphthalene	ND (1.0)	2.8	ND (1.4)	ND (1.5)
Biphenyl	ND (0.4)	ND (0.3)	ND (0.7)	ND (0.8)
2-Chloronaphthalene	ND (0.5)	ND ()0.4	ND (0.9)	ND (1.1)
2,6 & 2,7-Dimethylnaphthalene	3.9	5.6	ND (1.1)	12.6
1,2-Dimethylnaphthalene	ND (0.6)	ND (0.5)	ND (1.1)	ND (1.4)
Acenaphthylene	ND (0.4)	ND (0.3)	ND (0.7)	ND (0.8)
Acenaphthene	ND (0.5)	ND (0.4)	ND (0.9)	ND (1.1)
Fluorene	7.2	17.1	20.1	20.0
Phenanthrene	3.3	6.8	ND (0.8)	ND (0.9)
Anthracene	ND (0.7)	ND (0.5)	ND (1.1)	ND (1.2)
o-Terphenyl	ND (0.9)	ND (0.5)	ND (1.2)	ND (1.3)
m-Terphenyl	ND (0.7)	ND (0.4)	ND (1.0)	ND (1.0)
p-Terphenyl	ND (0.6)	ND (0.3)	ND (0.9)	ND (0.9)
2-Methylanthracene	ND (0.6)	ND (0.3)	ND (0.9)	ND (0.9)
9-Methylphenanthrene	ND (0.5)	ND (0.3)	ND (0.7)	ND (0.7)
1-Methylphenanthrene	ND (0.5)	ND (0.3)	ND (0.8)	ND (0.8)
Fluoranthene	ND (0.3)	ND (0.2)	ND (0.5)	ND (0.5)
Pyrene	ND (0.3)	ND (0.2)	ND (0.5)	ND (0.5)
9,10-Dimethylanthracene	ND (1.3)	ND (0.8)	ND (1.9)	ND (1.9)
Benzo(a)fluorene	ND (0.9)	ND (0.5)	ND (1.3)	ND (1.3)
Benzo(b)fluorene	ND (1.0)	ND (0.6)	ND (1.4)	ND (1.4)
Benz(a)anthracene	ND (0.5)	ND (0.4)	ND (0.7)	ND (0.8)
Triphenylene/Chrysene	ND (0.6)	ND (0.5)	ND (0.9)	ND (1.1)
Benz(b)anthracene	ND (2.5)	ND (2.1)	ND (4.0)	ND (4.7)
5-Methylchrysene	ND (1.4)	ND (1.2)	ND (2.3)	ND (2.7)
1-Nitropyrene	ND (21.5)	ND (18.1)	ND (34.5)	ND (40.6)
7,12-Dimethylbenz(a)anthracene	ND (3.4)	ND (2.8)	ND (5.4)	ND (6.3)
Benzo(b)fluoranthene	ND (0.7)	ND (0.7)	ND (1.9)	ND (1.6)
Benzo(k)+(j)fluoranthene	ND (1.0)	ND (1.0)	ND (2.5)	ND (3.3)
Benzo(e)pyrene	ND (1.2)	ND (1.0)	ND (2.5)	ND (2.0)
Benzo(a)pyrene	ND (1.3)	ND (1.1)	ND (2.7)	ND (2.2)
Perylene	ND (1.8)	ND (1.2)	ND (3.3)	ND (2.6)
3-Methylcholanthrene	ND (2.1)	ND (1.4)	ND (3.9)	ND (3.0)
Indeno-(123cd)pyrene	ND (0.1)	ND (0.2)	ND (0.4)	ND (0.5)
Benzo(g,h,i)perylene	ND (0.2)	ND (2.7)	ND (0.6)	ND (0.7)
Dibenz(ac)+(ah)anthracene	ND (0.2)	ND (0.3)	ND (0.5)	ND (0.7)
Picene	ND (0.4)	ND (6.2)	ND (1.5)	ND (1.7)
Coronene	ND (0.4)	ND (6.5)	ND (1.5)	ND (1.7)
Dibenz(a,e)fluoranthene	ND (0.8)	ND (12.3)	ND (2.9)	ND (3.3)
Dibenz(a,l)pyrene	ND (0.5)	ND (7.9)	ND (1.9)	ND (2.1)
Dibenz(a,e)pyrene	ND (0.7)	ND (10.1)	ND (2.4)	ND (2.7)
Dibenz(a,i)pyrene	ND (1.4)	ND (20.8)	ND (4.9)	ND (5.5)
Dibenz(a,h)pyrene	ND (2.3)	ND (33.9)	ND (8.0)	ND (9.0)
Dibenz(a,h)acridine	ND (0.5)	ND (7.9)	ND (1.8)	ND (2.1)
Dibenz(a,j)acridine	ND (0.5)	ND (6.7)	ND (1.6)	ND (1.8)
7H-dibenzo(c,g)carbazole	ND (0.6)	ND (8.8)	ND (2.1)	ND (2.3)

*not detected (limit of detection)

2.11.3. Dioxins and Furans

Congener	Dioxin/Furan Concentration (ppt)			
	753	754	755	756
Dioxins				
2,3,7,8-TCDD	ND (0.1)[1]	ND (0.1)	ND (0.1)	ND (0.1)
Total TCDDs	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
1,2,3,7,8-PeCDD	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
Total PeCDDs	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
1,2,3,4,7,8-HxCDD	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
1,2,3,6,7,8-HxCDD	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
1,2,3,7,8,9-HxCDD	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
Total HxCDDs	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
1,2,3,4,6,7,8-HpCDD	ND (0.3)	0.3	ND (0.4)	ND (0.4)
Total HpCDDs	ND (0.3)	0.3	ND ()0.4	ND (0.4)
OCDD	ND (1.0)	0.9	ND (0.6)	ND (0.8)
Furans				
2,3,7,8-TCDF	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
Total TCDFs	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
1,2,3,7,8-PeCDF	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
2,3,4,7,8-PeCDF	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
Total PeCDFs	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
1,2,3,4,7,8-HxCDF	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
1,2,3,6,7,8-HxCDF	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
2,3,4,6,7,8-HxCDF	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
1,2,3,7,8,9-HxCDF	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
Total HxCDFs	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
1,2,3,4,6,7,8-HpCDF	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
1,2,3,4,7,8,9-HpCDF	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
Total HpCDFs	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
OCDF	ND (0.3)	ND (0.3)	ND (0.3)	ND (0.5)
WHO TEQ[2]	0.340	0.340	0.341	0.341

1. ND = not detected (limit of detection)
2. World Health Organization Toxic Equivalent

3. INTENDED USE AND CONSUMER EXPOSURE

3.1 Intended Technical Effect

The intended technical effect of the addition of whole or milled flaxseed to conventional foods is to provide both α-linolenic acid (ALA) and dietary fiber in order to increase the intake of these nutritional components. These effects are consistent with the definition of nutrient supplements in 21 CFR §170.3(o)(20).

Whole or milled flaxseed may also be added to foods, including meat and poultry products at up to 3.5% by weight, as binders [§170.3(o)(14)].

The addition of whole or milled flaxseed to foods may also provide other technical effects including stabilizing, thickening, or bulking [§170.3(o)(28)] and texturizing [§170.3(o)(32)].

3.2. α-Linolenic Acid

3.2.1. Recommended Intake of α-Linolenic Acid

As was discussed earlier, α-linolenic acid (ALA) is an essential fatty acid because it is a precursor to the formation of EPA and DHA. The IOM Panel on Macronutrients (IOM 2002) was not able to establish sufficient quantitative information regarding ALA requirements to set a Recommended Dietary Allowance (RDA); however, it did establish Adequate Intakes (AI) based on median intakes of ALA in the U.S., where ALA deficiency is essentially nonexistent. The AIs for various age/sex groups are shown below in Table 28.

Table 28. Adequate Intakes of α-Linolenic Acid.

Sex/Age Category	AI[1] for α-Linolenic Acid (g/day)[2]
M&F, 1-3 years	0.7
M&F, 4-8 years	0.9
M, 9-13 years	1.2
F, 9-13 years	1.0
M, 14->70 years	1.6
F, 14->70 years	1.1
Pregnant	1.4
Lactating	1.3

[1]AI = Adequate Intake
[2]Source: IOM (2002), pp. 8-37 through 8-39.

Since flaxseed contains about 25% ALA, these requirements can be achieved by ingesting 4.4 g/day of flaxseed for an adult female and 6.4 g/day for an adult male; lesser amounts will provide the required ALA intake for children.

Gebauer et al. (2006) surveyed the literature regarding the role of n-3 fatty acids and cardiovascular benefits and noted that 6 authoritative bodies had promulgated recommended daily intakes of ALA. Among these bodies, the IOM committee's AI (which the IOM established as an intake that merely appears to be protective against clinical deficiency, and not an optimal intake to achieve maximum benefit) was the lowest number. Higher recommended daily intakes of ALA were recommended by:

- a North Atlantic Treaty Organization workshop in 1989 (recommended 3 g ALA/day);
- a workshop of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) in 1999 (recommended 2.2 g ALA/day);
- the Eurodiet Commission in 2000 (recommended 2 g ALA/day);
- the French Apports Nutritionnels Conseilles in 2001 (recommended 1.8 g ALA/day); and
- ISSFAL in 2004 (recommended 1.6 g ALA/day).

3.2.2. Current and Adequate Intake of α-Linolenic Acid

Since the AI was selected as the median intake of ALA by various age/sex groups in the U.S. population, average intakes are about the same as the AI. The primary sources of ALA in the U.S. diet (Gebauer et al. 2004) are grain products (0.41 g ALA/day for adult males, 0.29 g/day for adult females); meat, poultry, and fish (0.38 and 0.23 g/day); fats and oils (0.34 and 0.28 g/day); and vegetables (0.23 and 0.17 g/day). Clearly, however, half of adults in the U.S. have less than the median intake of ALA and these individuals would benefit from increasing their consumption of foods containing ALA.

3.3. Fiber

3.3.1. Definitions of Fiber

Prior to 2001, the situation regarding labeling and defining dietary fiber in the United States and many other countries was arbitrary due to its reliance on analytical methods as opposed to an accurate definition that includes its role in health (IOM 2001). For this reason, the Institute of Medicine's (IOM) Food and Nutrition Board assembled a Panel on the Definition of Dietary Fiber. This panel recommended defining "dietary fiber" as "nondigestible carbohydrates and lignin that are intrinsic and intact in plants" (IOM 2001, p 2). It then suggested defining "added fiber" as "isolated, nondigestible carbohydrates that have beneficial physiological effects in humans" (IOM 2001, p 2). Finally, the panel defined "total fiber" as the sum of dietary fiber and added fiber (IOM 2001).

With regard to the traditional segregation of dietary fibers into soluble and insoluble varieties, the panel concluded:

"It is recommended that the terms soluble and insoluble fiber be phased out and replaced with the appropriate physicochemical property as the characterization of the properties of various fibers becomes standardized" (IOM 2001, p 25).

It is noteworthy that the IOM panel emphasized consideration of physicochemical properties and strongly urged the inclusion of a requirement that a substance must provide a beneficial physiological effect in humans in order to be regarded as an added fiber. This condition would appear to exclude isolated non-digestible carbohydrates with no demonstrated benefits from being defined as fibers. Perhaps for this reason, the IOM's Panel on Macronutrients used the term "functional fiber" in place of the earlier panel's term "added fiber" (IOM 2002) in discussing recommended and safe levels of intake of total fiber.

While the 2001 panel did not attempt to restrict the range of physiological benefits that might be demonstrated as being provided by putative fiber substances, it specifically described three "established physiological effects" that are frequently characteristic of added fibers (IOM 2001). These are:

- attenuation of the postprandial blood glucose response,
- attenuation of blood cholesterol concentrations, and
- improved laxation.

It is probably not a coincidence that these effects are the same as those identified in 1988 by Health Canada (Health Canada 1988). The panel did not suggest that a substance needs to provide all three of these benefits in order to be regarded as an added fiber (IOM 2001), nor did it exclude defining a substance as an added fiber based on an ability to confer benefits other than these; the intention was to require that a substance could not be defined as an added fiber unless it was shown to provide a benefit.

Following the IOM panel's recommendation, in supporting the intended use of flaxseed as a source of dietary fiber it is appropriate to consider the extent to which it has been shown to provide physiological benefits. This matter is addressed below in the course of the discussion of human studies involving flaxseed or its constituents.

3.3.2. Recommended Intake of Dietary Fiber

To estimate the human requirement for fiber, the Panel on Macronutrients (IOM 2002) reviewed a large body of research relating fiber intake to a number of health endpoints. These included reduction in the risk of hyperlipidemia, hypertension, and coronary heart disease; gastrointestinal health, including duodenal ulcers, constipation, laxation, fecal weight, production of short-chain fatty acids, and diverticular disease; colon cancer, breast cancer, and other cancers; glucose tolerance and insulin response; and satiety and weight maintenance. The panel elected to use the level of fiber intake needed to achieve significant reduction in the risk of coronary heart disease as the basis for establishing a minimum human requirement.

Data were lacking to set an estimated average requirement (EAR) because the benefit of elevated total fiber intake occurs continuously across the whole range of intakes against which

impact on the advent of coronary heart disease is now known from prospective studies (IOM 2002). The Panel noted:

> "Because the available evidence suggests that the beneficial effects of fiber in humans are most likely related to the amount of food consumed, not to the individual's age or body weight, the best approach is to set an Adequate Intake (AI) based on g/1000 kcal" (IOM 2002, p 7-34).

Based on the average of the reviewed studies on dietary fiber and coronary heart disease, and the beneficial role of functional fibers, the Panel set the AI for total fiber at 14 g/1000 kcal. The AI was set on this basis, rather than on a g/kg or g/day basis, because "the evidence suggests that the beneficial effects of fiber in humans are most likely related to the amount of food consumed" (IOM 2002, p 7-34). The Panel considered that there is no reason to believe that fiber intake as a function of energy intake differs during the life cycle; thus, AIs for various sex/age groups were determined by multiplying [14 g/1000 kcal] X [median energy intake of each group]. The results of these calculations are shown in Table 29.

Table 29. Median Energy Intakes and Adequate Intakes of Total Fiber.

Sex/Age Category	Median Energy Intake (kcal/day)[2]	AI[1] for Total Fiber (g/day)[3]
M&F, 1-3 years	1372	19
M&F, 4-8 years	1759	25
M, 9-13 years	2226	31
F, 9-13 years	1877	26
M, 14-18 years	2748	38
F, 14-18 years	1872	26
M, 19-30 years	2718	38
F, 19-30 years	1757	25
M, 31-50 years	2476	38
F, 31-50 years	1659	25
M, 51-70 years	2109	30
F, 51-70 years	1507	21
M, >70 years	1773	30
F, >70 years	1356	21
Pregnant	1978	28
Lactating	2066	29

1. AI = Adequate Intake
2. Source: IOM (2002), Table E-1; data from USDA (2000)
3. Source: IOM (2002), pp. 7-36 through 7-39.

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3.3.3. Current Intake of Dietary Fiber and Adequate Intake of Total Fiber

Comparison of the actual intake of dietary fiber with the adequate intake (AI) of total fiber indicates the gap that might be filled by functional fiber. This comparison is shown in Table 30, where intakes of dietary fiber at the 10th, 50th, and 90th percentiles are compared with the AI for total fiber.

Table 30. Gaps Between Usual and Adequate Intakes of Fiber.

Sex/Age Category	Gap Between Usual Intake of Dietary Fiber and AI for Total Fiber (g/day)[1]		
	Gap From 10th Percentile	Gap from 50th Percentile	Gap from 90th Percentile
M&F, 1-3 years	13.4	9.9	5.2
M&F, 4-8 years	16.7	13.2	8.6
M, 9-13 years	21.1	16.4	9.8
F, 9-13 years	17.2	13.4	8.4
M, 14-18 years	26.9	21.0	13.0
F, 14-18 years	17.4	13.5	8.5
M, 19-30 years	27.9	20.6	9.8
F, 19-30 years	17.4	12.9	6.6
M, 31-50 years	27.1	20.1	10.0
F, 31-50 years	17.3	11.9	4.3
M, 51-70 years	20.1	12.5	1.7
F, 51-70 years	12.7	7.2	0
M, >70 years	21.0	13.5	2.7
F, >70 years	13.4	7.7	0
Pregnant	17.8	12.4	4.9
Lactating	18.0	10.7	0.1

1. Source of data: IOM (2002), using data from USDA (2000)

It is evident that there are significant gaps between recommended levels of intake of total fiber and current levels of intake of dietary fiber. These gaps can be filled only by significant increases in consumption of fiber-containing foods or by the addition of functional fiber to foods already being consumed. Even individuals at the 90th percentile of consumption of dietary fiber may fall 10 g or more below recommended levels of daily fiber intake; for those at the other end of the consumption distribution, the 10th percentile of consumption, the gap between intake and recommendation may approach 30 g/day.

These data demonstrate that there is room for a substantial increase in intake of total fiber before the possibility of overconsumption even arises: the first 10-20 g/day of added intake merely suffice to bring the average individual up to recommended levels.

3.4. Intended Addition of Whole and Milled Flaxseed to Foods

Flaxseed, whether whole or milled, is intended for addition to conventional foods with its use limited only by cGMP, standards of identity, or other legal constraints, with the exception of its use as a binder in meat and poultry products, for which its level will not exceed 3.5% by weight.

3.5. Estimated Intakes of Whole and Milled Flaxseed

Although the addition of flaxseed to foods is intended to be limited only by cGMP, there are both technological limitations and issues of organoleptic characteristics of food that restrict both the variety of foods to which flaxseed is actually added and the addition level. As was noted by the IOM (2002) panel, "... due to the bulky nature of fibers, excess consumption is likely to be self-limiting" (p 7-46). Thus, any reasonable estimate of the likely daily intake of flaxseed and fiber from the intended uses of flaxseed must take these restrictions into account.

As discussed in Section 5.2, FDA previously authorized the addition of flaxseed to foods at up to 12% by weight, and this may be taken as a representative maximum addition level for most foods. While it may be technologically and organoleptically feasible to add a somewhat higher proportion of flaxseed to a few food products, for many products even 12% flaxseed addition is likely to be unattainable. Thus, estimating that target foods may have 12% maximum addition of flaxseed provides a conservative basis for estimating likely potential exposure. A representative selection of target foods for flaxseed addition is shown below in Table 31. Using the Reference Amounts Customarily Consumed (RACC) established by FDA (21 CFR §101.12), addition levels of 12% by weight can be converted to g/serving by multiplying the RACC by the intended addition percentage. The results of these calculations are shown in the last column of Table 31.

With the exception of yogurt, for which it may be doubted that addition of 12% flaxseed would be organoleptically acceptable, the range of maximum intended addition does not exceed 6.6 g/serving, with a modal level of 3.6 g/serving. A reasonable approximation of the average maximum intended level of flaxseed addition is 4 g/serving. This figure is an extremely conservative but reasonable estimate of the average amount of flaxseed that may be added to foods when such addition is restricted only by technological and organoleptic limitations.

In estimating the intake of flaxseed, fiber, and α-linolenic acid (ALA), then, it is assumed that flaxseed is added to foods at an average of 4 g/serving. Since flaxseed is about 25% fiber and about 25% ALA, the average added fiber content of these foods is 25% of 4 g, or 1 g/serving, and the average added ALA content is the same, 1 g/serving.

Table 31. Intended Addition Levels of Flaxseed by Weight and Per Serving.

Food Category	RACC[1] (g)	Maximum Flaxseed Content[2] (g/serving)
Breads	50	6.0
Biscuits, bagels, tortillas	55	6.6
Doughnuts, muffins	55	6.6
Pancake/waffle mix	40	4.8
Pasta (dry weight)	55	6.6
Multigrain flours or meal	30	3.6
Multigrains	45	5.4
Breakfast cereals (high fiber)	30	3.6
Cookies	30	3.6
Pizza crust	55	6.6
Snack bars (grain based)	40	4.8
Batter mixes, breading	30	3.6
Crackers	30	3.6
Popcorn, chips, pretzels	30	3.6
Nuts and seeds	30	3.6
Cheeses	30	3.6
Salad dressings	30	3.6
Mayonnaise	15	1.8
Margarine, table spreads	15	1.8
Yogurt	225	27.0

[1]Reference Amount Customarily Consumed (21 CFR §101.12).
[2]Based on addition of flaxseed at 12% by weight of the food

Because flaxseed may be added to a wide variety of foods but is unlikely to be added to all of them, and because its bulking effect limits the amount that consumers are likely to ingest per day, using food-consumption survey data to estimate mean and 90th percentile food consumption and muliplying by flaxseed addition does not provide reasonable estimates of daily intake. However, FDA has previously dealt with estimating fiber intakes in developing the final rule regarding health claims for β-glucan soluble fibers from oats and psyllium (FDA 1997a) and in the health claim for soy protein. The Agency was required to promulgate a rule for the minimum amount of β-glucan fiber from oats or psyllium, or the amount of soy protein, that a food must contain in order to use the approved health claim. Obviously, each rule would have to bear a reasonable relationship to the amount of β-glucan fiber from oats or psyllium or the amount of soy protein that is needed to provide the claimed benefit. FDA determined that the minimum content in order to use the health claim would be ¼ of the amount of the substance

needed to obtain the benefit on the assumption that individuals attempting to obtain the benefit would be able to consume about 4 servings per day of foods containing β-glucan soluble fiber from oats or from psyllium or containing soy protein (Health claim: soluble fiber from certain foods and risk of coronary heart disease, 21 CFR §101.81; Health claim—soy protein and risk of coronary heart disease, 21 CFR §101.82). The Agency explained its reasoning in some detail in the final rule (FDA 1997a) authorizing the health claim for β-glucan soluble fiber from oats:

> "Many comments stated that the qualifying level of β-glucan soluble fiber per serving should not be based on three servings of oat products per day but rather on FDA's usual basis of four eating occasions (three meals and a snack) a day.
>
> "As discussed previously, an intake of 3 or more g of β-glucan soluble fiber from whole oat products is necessary to make a significant impact on serum lipid levels. Using the minimum levels of β-glucan soluble fiber for oat bran (5.5 percent) and rolled oats and whole oat flour (4 percent) ..., products that contain a minimum of 0.5 g β-glucan soluble fiber would contain about 9 g of oat bran or 12.5 g rolled oats or whole oat flour, or a level between 9 and 12 g if a blend of whole oats is used. To obtain a daily intake of 3 g β-glucan from whole oats, it would require the consumption of six or more servings. Similarly, if the oat products qualified with 0.6 g β-glucan soluble fiber, consumers would have to consume five or more servings of oat-containing products daily. The agency finds that these levels of consumption, five or six or more servings per day, are highly unlikely. As mentioned in some of the comments, consumers should be able to consume a beneficial amount of the nutrient based on typical American eating patterns, i.e., four eating occasions per day.
>
> "In the proposal, the agency considered the number of eating occasions at which consumers might consume oat bran and rolled oats. The agency tentatively agreed with the petitioner's arguments that it was unlikely that consumers would eat oat bran or rolled oats 4 times a day, in order to consume a daily intake of about 40 g oat bran or 60 g rolled oats, but that consumers should be able to consume this amount over three eating occasions a day (61 FR 296 at 309). Based on the petitioner's submission, the agency considered that β-glucan soluble fiber would come from only two sources, oat bran and rolled oats, which would limit the number and types of products available.
>
> "In this final rule, however, the agency has expanded the sources of whole oats to include whole oat flour. Thus, many more whole oat-containing products will be available to qualify to bear this claim. This development increases the likelihood that whole oat products will be consumed at four, instead of three, eating occasions. Moreover, based on consumption data provided in a comment submitted by the petitioner, whole oat products (including all oat cereals, baked products, and snack foods) are consumed at four eating occasions a day, with breakfast being the most popular time to consume oat products (see Sup-1 to Docket No. 95P-0197). Therefore, based on the expanded focus of this final regulation (to include whole oat flour) and on the additional evidence from

comments, the agency is persuaded that the determination of the qualifying level of β-glucan for a food to bear a claim should be based on four eating occasions a day (three meals plus a snack) rather than on the proposed three" (p 3592).

Based on this FDA model, the estimated daily intake (EDI) of flaxseed added at 4 g/serving to a variety of foods is 16 g/day[1]. As a dietary concentration, 16 g is 0.5% of a 3 kg/day total diet. The EDI of functional fiber and of ALA provided by flaxseed are 25% of the EDI for flaxseed, or 4 g/day. Since in many cases the addition level of flaxseed is less than 4 g/serving, and since it may be doubted that most individuals will regularly consume flaxseed-containing foods 4 or more times per day, the EDI is likely to be less than 16 g/day and the EDI of fiber and ALA are proportionately reduced.

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[1] Note that this is a conservative estimate. FDA raised the number of likely eating occasions for whole oats from 3 to 4 only after the number of potential sources of whole oats was increased from 2 to 3. Since there is only 1 source of flaxseed, it may actually be more congruent with FDA's thinking to estimate 3 rather than 4 eating occasions a day, or an EDI of 12 g rather than 16 g.

4. REVIEW OF SAFETY DATA

4.1. Kinetics and Metabolism of Flaxseed

Flaxseed contains about 25% oil composed primarily (40-60%) of α-linolenic acid (ALA), an omega-3 fatty acid, varying amounts of the lignan secoisolariciresinol diglycoside (SDG), and protein, as well as mucilage and cellulose which are neither absorbed nor digested. Studies investigating the absorption, distribution, metabolism, and excretion (ADME) of flaxseed typically examine the kinetics of two of its main components, ALA and SDG.

4.1.1. α-Linolenic Acid

The metabolism of ALA has been studied extensively and was recently reviewed by Burdge (2006). Figure 12, taken from this review, shows the metabolic fate of ALA in humans.

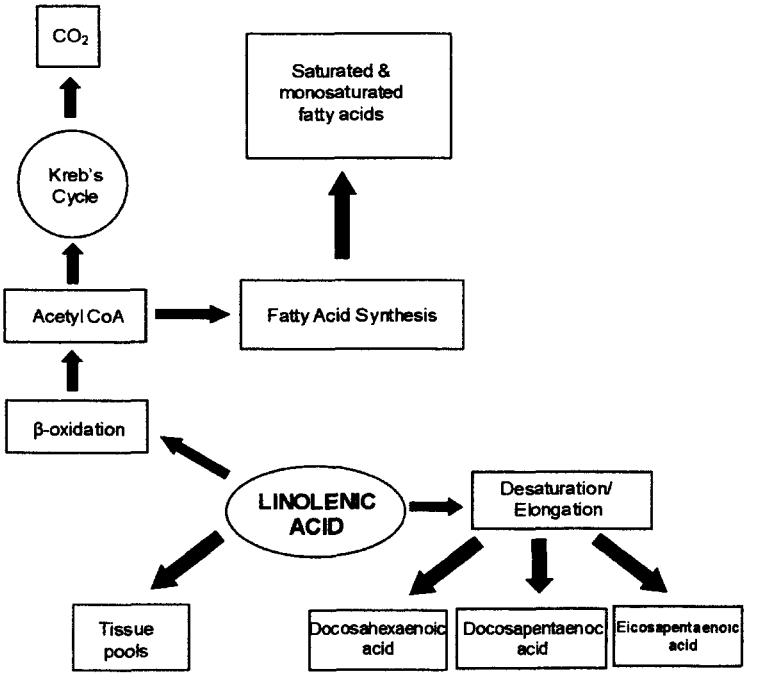

Figure 12. Metabolic Fate of α-Linolenic Acid in Humans (from Burdge 2006)

Orally ingested ALA is almost completely absorbed from the gastrointestinal tract. A single individual who ingested 750 mg [U-^{13}C] ALA was shown to absorb >96% of the administered dose over a 5-day period (Burdge 2006). Cunnane et al. (1993) assessed the bioavailability of the ALA in ground flaxseed. In a randomized crossover study, 5 healthy females (average age 20) consumed either 20-g flaxseed oil capsules or 50 g/day flaxseed flour

for 4 weeks with a 2-week washout period in between. Both treatments provided 20 g/day of flaxseed oil containing 12 g/day of ALA. Venous blood samples were collected at 2-week intervals for analysis of plasma total n-3 fatty acids and ALA. Changes in both total n-3 fatty acids and ALA were equivalent, suggesting that the ALA in ground flaxseed is as well absorbed as it is from flaxseed oil, although the absolute levels of absorption were not estimated.

Once absorbed, there are 3 potential metabolic pathways that ALA can follow: 1) pooling in adipose tissue; 2) β-oxidation; or 3) desaturation/elongation. These are briefly discussed below.

ALA is estimated to account for ~0.7% of the total fatty acids in neutral lipids in human adipose tissue (Tang et al. 1993; Kaminskas et al. 1999). Six hours after ingestion of an unspecified dose of [^{13}C]-ALA, 15-81% of the radioactivity was detected in adipose tissue of healthy female subjects (McCloy et al. 2004).

Up to one third of administered ALA has been reported to follow fatty acid β-oxidation pathways in male and female subjects, although fractional oxidation of [^{13}C]-ALA tended to be less in women (22%) than in men (33%) over a similar sampling period (DeLany et al. 2000; Vermunt et al. 2000; Bretillon et al. 2001; Burdge and Wootton 2002; Burdge et al. 2002, 2003). Once carbon is released from ALA through β-oxidation, it enters the acetyl-CoA pool and then may either enter the Kreb's cycle and be expired as CO_2, or be used in fatty acid synthesis. Men were reported to have 20% higher total concentrations of labeled saturated and monounsaturated fatty acids in plasma lipids than women (Burdge and Wootton 2003).

ALA can also be metabolized in the liver to form eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) via the desaturation/elongation pathway (Sprecher 2002). Tracer studies in human subjects have shown variations in the blood concentration of labeled ALA metabolites (Burdge 2004; Burdge and Calder 2005): EPA (0.2% to 6%), DPA (0.13% to 6%), and DHA (≤0.05%). Conversion of ALA to EPA or DHA was reported to be higher in women (2.5-fold and >200-fold, respectively) than in men (Burdge 2002). It has been suggested by Burdge (2006) that estrogen may play a role in increasing the activity of the desaturation/elongation pathway since women on oral contraceptives containing 17α-ethylnyloestradiol showed a 3-fold higher synthesis of DHA than did women not on oral contraceptives (Burdge and Wootton 2002).

4.1.2. Lignan: Secoisolariciresinol Diglycoside (SDG)

Colonic bacteria convert lignans such as secoisolariciresinol diglycoside (SDG) into the enterolignans enterodiol and enterolactone (see Figure 13), which then are absorbed, enter the blood circulation, and are eliminated via urine and feces. The metabolic pathways and the involved organs are similar in rats and humans, although the metabolic conversion rate appears to be faster in rats than in humans (Niemeyer et al. 2003). Enterolignans, which are also referred to as mammalian lignans, are of particular interest since it has been reported that they have antioxidant and (anti-)estrogenic effects, can induce NADPH:quinone reductase (phase II enzymes) and can inhibit enzymes such as 5α-reductase, sex hormone binding globulin, and 17β-hydroxysteriod dehydrogenase which are involved in the metabolism of sex hormones

(Adlercreutz et al. 1987; Welshons et al. 1987; Mousavi and Adlecreutz 1992; Evans et al. 1995; Schottner et al. 1997; Wang et al. 1998; Kitts et al. 1999; Prasad 2000).

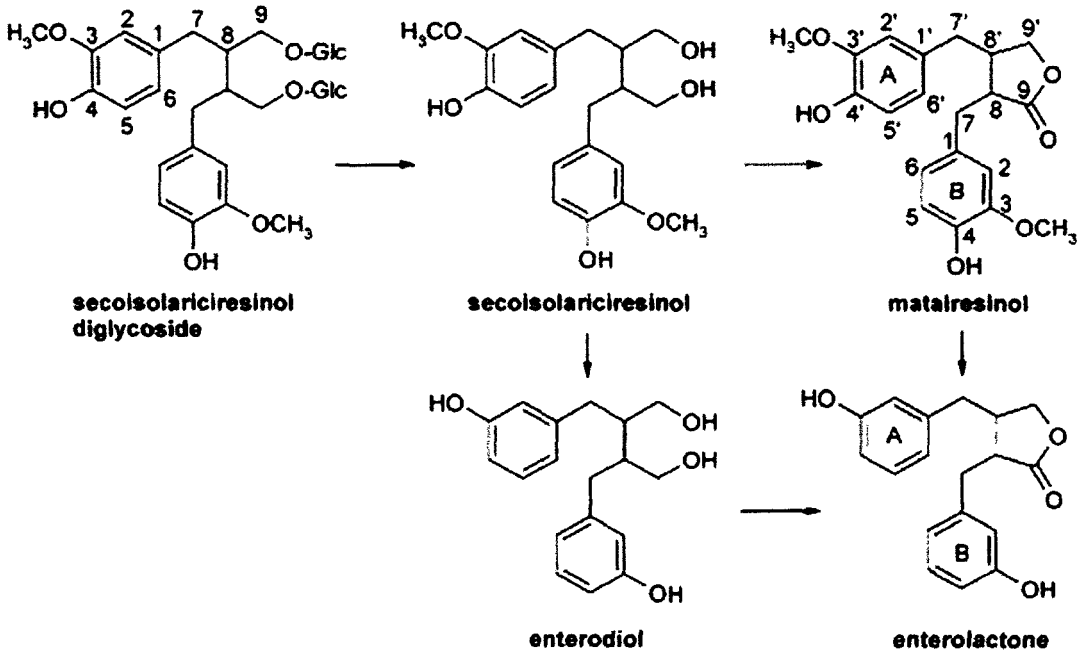

Figure 13. Metabolism of Secoisolariciresinol Diglucoside (SDG) by Colonic Bacteria (from Niemeyer et al. 2003)

4.1.2.1. Animal Studies

Female Sprague-Dawley rats (6/group) were fed a basal diet[1] supplemented with 0, 2.5, 5, or 10% ground flaxseed or gavaged daily with 1.1, 2.2, or 4.4 µmol purified SDG/ml saline (estimated to be equivalent to the SDG content of the flaxseed supplementation) for 2 weeks (Rickard et al. 1996). Twenty-four-hour urine samples were taken for 3 days after 1 week of treatment and analyzed for content of the enterolignans enterodiol, enterolactone, and secoisolariciresinol. Urinary excretion of total lignans from both the flaxseed and purified SDG groups increased linearly to the mid-dose but then showed a plateau between the mid and highest dose. Unmetabolized SDG (secoisolariciresinol) accounted for the lowest percentage of lignans excreted in urine (8-10% in flaxseed-fed animals and up to 32% in secoisolariciresinol diglycoside-fed animals) and followed a dose-response in animals fed flaxseed. Animals fed purified SDG followed a curvilinear pattern to the mid-dose after which urinary levels decreased. Total urinary lignan excretion was much lower in SDG-fed animals (0.2-0.4 µmol/24 hours) than in flaxseed fed animals (0.6-1.6 µmol/24 hours). Percent urinary excretion of total lignans was calculated using the HPLC values of SDG and were 74.26±17.41, 92.10±11.57, and

[1] The basal diet was based on the AIN-76 formulation except that a higher level of fat was provided by the addition of corn oil at the expense of cornstarch.

46.25±7.10% for the 2.5, 5, and 10% flaxseed diet groups, respectively. The reduction of urinary excretion of lignans at the highest dose may be due to increased body retention or increased fecal excretion.

In the same study, an *in vitro* fermentation experiment was conducted using triplicate samples of 1.1, 2.2, and 4.4 μmol of purified SDG or 0.5 g flaxseed fermented with human fecal inoculum in order to simulate human colonic microfloral conditions (Rickard et al., 1996). After 24 hours, the fermentation was stopped with copper sulfate and samples were centrifuged and filtered. The filtrate was analyzed for lignan content. Production of enterodiol and enterolactone was higher than that of the *in vivo* study; however, secoisolariciresinol levels were negligible. Similar to the *in vivo* study, enterodiol and enterolactone levels were linear with dietary concentration up to the mid-level and then leveled off to the highest concentration. Percent urinary excretion of total lignans in the *in vivo* study were calculated using the *in vitro* fermentation values and were 58.70±12.31, 72.81±8.35, and 36.56±5.12% for the 2.5, 5, and 10% flaxseed diet groups, respectively.

To examine the body disposition and excretion of orally ingested SDG, Rickard and Thompson (1998) gavaged a group of 12 female 70-72-day-old Sprague-Dawley rats (acute group) with a single dose of ^{3}H-SDG following an overnight fast, placed the rats in individual metabolic cages for collection of urine and feces, and euthanized them at 12, 24, 36, or 48 hours post treatment (3 rats/time point). Another group of 12 rats (chronic group) was first gavaged daily with 1.5 mg unlabeled SDG for 10 days and then treated in the same manner as the acute group. Radioactivity was determined by scintillation counting in urine, blood, GI content, feces, and tissues. More than 50% of the recovered dose was excreted in the feces by 12 hours in rats from the acute group and by 24 hours in rats from the chronic group. At 12 hours, urinary excretion was 7% (acute group) and 3% (chronic group) of the recovered dose and at 48 hours reached 28-32% (both groups), indicating no differences in urinary radioactivity between acute and chronic exposures. Cecum and colon content represented 27.5% (acute group) and 57% (chronic group) of the recovered dose at 12 hours. By 48 hours, the cecum and colon radioactivity levels dropped to 1-2% of the recovered dose in both groups. The cecum had the highest level of radioactivity over 48 hours and decreased over time as did other GI tissues. Other tissues represented ~4-6% of the recovered dose at 12 hours, decreasing to ~3% by 48 hours. Excluding GI tissues, the highest levels of radioactivity were detected in the liver, kidney, and uterus. Notable differences between the acute and chronic tissue radioactivity levels included: liver levels were 50-80% higher in the chronic group than in the acute group over 48 hours; adipose levels were 3-fold higher in the chronic group at 12 hours, but were higher in the acute group at 36 hours; spleen levels were significantly higher in the chronic group at 36 hours; ovary levels were significantly higher in the acute group at 36 hours; and uterus levels were significantly higher in the chronic group at 12 hours. Plasma radioactivity was <1% throughout the entire study.

The metabolic profile of orally administered SDG was further investigated in 3 experiments using 70-72-day-old female Sprague-Dawley rats housed in individual metabolic cages (Rickard and Thompson 2000). In the first experiment, rats were treated as previously described in Rickard and Thompson (1998). Urine samples underwent extraction procedures to

isolate radioactive urinary lignan metabolites for HPLC analysis and scintillation counting. In addition, radioactive HPLC fractions underwent GC-MS analysis. Enterodiol, enterolactone, and secoisolariciresinol comprised 79.7±4.0% (acute group) and 73.8±7.7% (chronic group) of the total urinary radioactivity recovered over 48 hours. The ratio of enterodiol to enterolactone did not differ between acute and chronic groups. Three additional unknown peaks were detected by HPLC but were much smaller than peaks elicited by enterodiol, enterolactone, and secoisolariciresinol.

In the second experiment, urine collected for 3 days from rats fed a 5% flaxseed diet[1] or administered 1.5 mg SDG/day for 20 weeks was analyzed by GC-MS for any unknown lignan metabolites. Four unknown peaks were detected.

In the third experiment, groups of 3 (instead of 12) rats were treated as described in Rickard and Thompson (1998), with the exception that blood samples were collected from the tail vein at 0, 3, 6, 9, 12, and 24 hours after radiolabel treatment and underwent liquid scintillation counting for radioactivity measurements. Whole-blood radioactivity levels peaked at 9 hours for both treatment groups and remained higher than baseline at 24 hours.

4.1.2.2. Human Studies

In a randomized crossover study examining the metabolism of SDG in flaxseed, 9 healthy young women (aged 20-40 years) supplemented their diets with 5, 15, or 25 g of raw flaxseed or 25 g of processed flaxseed (in muffin or bread) for a 7-day period (Nesbitt et al. 1999). The SDG content of raw flaxseed was determined by HPLC to be 2.93 μmol/g. Consumption of flaxseed occurred during the follicular phase of the menstrual cycle because it had been previously reported that enterolactone urinary excretion increased significantly during the midluteal phase of the cycle (Setchell et al. 1980). Urine and blood samples were taken at selected intervals. Increased consumption of flaxseed led to significantly increased urinary excretion of enterolactone, enterodiol, and secoisolariciresinol. Total urinary lignan excretion ranged from 16-fold higher than baseline (5 g flaxseed supplementation) to 202-fold higher than baseline (25 g flaxseed supplementation). No significant differences in total urinary excretion were noted between 25 g processed or raw flaxseed.

As part of a second experiment, the same 9 young women ingested 25 g raw flaxseed for an additional day (i.e., 8 days rather than 7 days), and timed urine and blood samples were taken for analysis of enterolignan content. On day 1, urinary excretion of enterodiol, but not enterolactone or secoisolariciresinol, was significantly higher during the 12-24 hour period than the 0-12 hour period. By the 8th day, there was no difference between the 12-24 hour and 0-12 hour periods. For both enterodiol and enterolactone, urinary excretion was significantly greater during the 0-12 hour period on day 8 than the same period on day 1. Mean total urinary excretion of lignans (enterodiol + enterolactone + secoisolariciresinol) was significantly higher

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[1] The diet was based on the AIN-93G formulation except that a higher fat concentration was achieved by the addition of 20% soybean oil at the expense of cornstarch and dextrose.

on day 8 (233.35±87.41 μmol/24 hours) than on day 1 (47.67±21.04 μmol/24 hours). Plasma concentration of enterodiol + enterolactone was significantly increased by 9 hours post-ingestion on day 1 and did not change significantly at 12 or 24 hours. By the 8th day, plasma concentrations were significantly higher than at baseline. The ratio of enterodiol to enterolactone concentration in plasma ranged from 1.5 to 4.5 and in urine ranged from 3.5 to 19.2, indicating that plasma and urinary concentrations of enterodiol are higher than enterolactone following flaxseed consumption.

The bioavailability of lignans from flaxseed has been shown to vary with the physical state (whole, crushed, or ground) of the flaxseed (Kuijsten et al. 2005a). In a randomized crossover study, 12 healthy human subjects (6 of each sex aged 18-64 years; mean age = 25 years) were given 300 mg/kg bw/day of whole, crushed, or ground flaxseed as supplements for 10-day intervals with 11-day washout periods between treatments. On the last day of each interval, venous blood samples were drawn and analyzed for enterodiol and enterolactone concentrations. Ground flaxseed ingestion resulted in the highest concentration (270±34 nmol/l) of plasma enterolignans, followed by crushed flaxseed (122±21 nmol/l), and whole flaxseed (85±19 nmol/l). This represents a 2- to 4-fold difference in relative bioavailability between whole and crushed or milled flaxseed, although it also demonstrates that SDG is available to a degree even from whole flaxseed. Enterolactone contributed the highest percentage (71-98%) to the total concentration of plasma enterolignans and was significantly higher during the whole flaxseed period (73%) and crushed flaxseed period (68%) than the ground flaxseed period (62%). Within-subject and between-subject variation was high—23-48% and 45-98%, respectively (Kuijsten et al. 2005a).

To examine the pharmacokinetics of ingested SDG, 6 men and 6 women aged 18-25 years were given a single oral dose of 1.31 μmol/kg bw of purified SDG following an overnight fast (Kuijsten et al. 2005b). Blood samples were taken immediately prior to treatment (0 hour) and every 3 hours for 36 hours and then at 48, 72, and 96 hours post-treatment. Urine samples were collected for 24 hours prior to treatment and for 72 hours post-treatment. Enterolactone and enterodiol were detected in plasma at 8-10 hours post-treatment with peak plasma concentrations occurring at 14.8±5.1 hours for enterodiol and 19.7±6.2 hours for enterolactone. Systemic exposure to enterolactone was approximately twice that of enterodiol as represented by the area under the curve (AUC). The mean elimination half-life for enterodiol and enterolactone was 4.4±1.3 hours and 12.6±5.6 hours, respectively, and the mean residence time for enterodiol and enterolactone was 20.6±5.9 hours and 35.8±10.6 hours, respectively, indicating that the enterolignans accumulate in plasma and reach a steady state when consumed on a regular basis. Most of the enterolignans that were excreted via the urine did so within the first 2 days and by day 3 up to 40% of the consumed dose was excreted via the urine. On the day 3 sampling, enterolactone comprised 58% of the total enterolignans excreted. The authors reported substantial variation among subjects in terms of plasma concentrations and urinary concentrations of enterolignans. Several differences between the sexes were observed: onset of the plasma curve of both enterolignans tended to be earlier in females (not statistically significant), maximum concentrations of both enterolignans tended to be higher in females (not statistically significant), and the residence times of both enterolignans were shorter in females ($p < 0.05$).

4.1.3. Comparative Kinetics of ALA and SDG In Humans and Rats

The absorption and metabolism of ALA has been little studied in the rat, and consequently no comparison of the pharmacokinetics between rats and humans is possible. SDG, on the other hand, has been studied in both rats rats and humans. Although the absorption, metabolism, and excretion of SDG are highly variable, the paths appear to be similar in rats and humans. In both models the proportion of fecal excretion, denoting non-absorption, increases as the dose increases. With regard to metabolism, SDG is converted into the same lignans in rats and humans, primarily enterodiol and enterolactone. Urinary excretion occurs more quickly in rats than in humans, but in general the rat appears to be an appropriate model for the study of SDG.

4.2. Toxicological Studies of Flaxseed and Its Constituents

The safety studies of oral exposure to flaxseed or its constitutents discussed in Section 4.2 are summarized in Table 32 at the end of the section.

4.2.1. Acute Toxicity Studies

Roberts et al. (2006) determined that the LD_{50} of 7-hydroxymatairesinol (7-HMR), a dibenzylbutyrolactone plant lignan chemically related to the matairesinol found in flaxseed (differing only in the replacement of hydrogen with a hydroxyl group at carbon 7), is >2000 mg/kg bw. (7-HMR also occurs in flaxseed, but at a concentration of only 35 µg/100 g seed [Smeds et al. 2007]).

4.2.2. Subchronic Toxicity Studies

4.2.2.1. Whole Flaxseed

4.2.2.1.1. Rat

Male and female weanling Sprague-Dawley rats were fed ground flaxseed as a dietary admixture and evaluated for any possible adverse or nutritional effects (Ratnayake et al. 1992). Groups of individually housed rats were randomized by weight into 4 groups and fed diets containing 0 (control), 10, 20, or 40% ground flaxseed (approximately 0, 7500, 15,000, or 30,000 mg/kg bw/day, respectively) for 90 days. Each diet was adjusted to be approximately isocaloric and contain similar amounts of fat (14.8-17.5%) and fiber plus wood cellulose (12.8-13.2%). The number of rats receiving each diet was not stated, but is known to be greater than 6 because it was reported that on days 27, 59, and 89, samples of 6 rats from each group were placed in metabolic cages and overnight fasting urine samples were collected. The samples were analyzed for urine peroxides expressed as thiobarbituric acid reacting substances (TBARS). Each morning on days 41-44, feces were collected, volume and weight were recorded, and the feces from each group were pooled for determination of total fat, pH, and fiber fermentability. At the end of the study, rats were fasted overnight and killed by exsanguination under pentobarbital anesthesia. Blood samples were collected and analyzed for serum total ,HDL, and LDL

cholesterol, triacylglycerols, and glucose levels. The heart, liver, kidney, spleen, and white and brown adipose tissue from the subscapular region were removed and weighed.

Body weights, feed consumption, serum glucose levels, and both absolute and relative organ weights of rats fed flaxseed were not statistically significantly different from controls. Total and LDL cholesterol and triacylglycerol levels were significantly lower than control values in rats fed 20 or 40% flaxseed. HDL cholesterol levels and the ratio of LDL to HDL cholesterol were significantly lower than controls in rats fed 40% flaxseed. TBARS excretion in the urine was significantly higher in rats fed 40% flaxseed for 60 and 90 days when compared with controls. α-tocopherol levels in the liver, heart, and spleen ,and γ-tocopherol levels in the liver and spleen, of rats fed 40% flaxseed were significantly lower than those of controls. The dietary content of α-tocopherol was calculated to be 6.1, 5.8, 5.5, and 5.0 IU/100 g diet for the 0, 10, 20, and 40% flaxseed diets, respectively. Dietary flaxseed produced significant increases in ALA in adipose tissue and organs compared to controls. The dietary fiber in flaxseed was associated with increased fecal moisture and appeared to be mostly fermentable. Ratnayake et al. (1992) concluded that dietary concentrations of up to 40% flaxseed (approximately 21 g/kg bw/day) produced no adverse effects and may actually have beneficial effects in lowering serum lipids. They also noted that ALA in flaxseed was bioavailable and was readily converted to long-chain n-3 fatty acids. The concentration of 22:6 (n-3) in the liver increased 3-fold when flaxseed in the diet was increased from 0%, to I0%, but further increases did not proportionately raise the 22:6 (n-3) concentration. However, 20:5 (n-3) and 22:5 (n-3) increased with increasing dietary flaxseed. In the heart, the level of 20:5 (n-3) was not significantly elevated except in the 40% diet group. The no observed adverse effect level (NOAEL) for flaxseed in this study was the highest level tested, 40% dietary concentration, approximately equivalent to 30,000 mg/kg bw/day.

The antioxidant potential of dietary flaxseed was studied in weanling female Sprague-Dawley rats (10 rats/group) aged 24 days at onset of the study (Yuan et al. 1999). The rats were divided into 3 treatment groups: 1) basal diet (AIN-93G[1]); 2) basal diet plus 10% ground flaxseed (about 7500 mg/kg bw/day); and 3) basal diet with a daily gavage of 3.0 mg/ml SDG in distilled water (about 9 mg SDG/kg bw/day). The flaxseed diet was made isocaloric to the basal diet and the basal diet and flaxseed diet groups also received a daily gavage of distilled water. The rats were individually housed in hanging plastic cages. Feed intake was monitored daily and body weight gains were determined weekly throughout the study. At 42 days of age, rats were placed in metabolic cages for 3 days to collect urine for analysis of urinary mammalian lignans enterodiol and enterolactone and the aglycone of SDG, secoisolariciresinol). At 50 and 51 days of age, the rats were terminated by CO_2 asphyxiation and their livers were removed for analysis of tissue glutathione levels and the antioxidant enzymes glutathione peroxidase, glutathione reductase, and catalase.

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[1] The AIN-93 and AIN-93G diet, fed to rodents as the basal diet in many of the studies reported in this monograph, derives its carbohydrates in the form of cornstarch and sucrose, protein as casein, lipid as soybean oil, and dietary fiber as powdered cellulose. The only other ingredients are mineral and vitamin mixes and TBHQ as an antioxidant (Reeves 1997).

Growth, feed efficiency, and liver-to-body weight ratios did not differ between treated and control groups. Total urinary lignans were significantly higher in flax-fed rats (3.781±0.657 μmol/day) and SDG-treated rats (1.668±0.146 μmol/day) compared to controls (0.034±0.013 μmol/day). Hepatic glutathione levels and glutathione peroxidase and catalase activity were similar between treated and control animals. Yuan et al. (1999) suggested that there may be a direct antioxidant function of mammalian lignans in the liver. While the authors did not establish a NOAEL, no adverse effects were seen at an intake of SDG of 9 mg/kg bw/day or a flaxseed concentration of 10%, approximately corresponding to 7500 mg/kg bw/day of ground flaxseed.

A study to examine the effects of 100 days of consumption of dietary oilseed on the liver and hepatic γ-glutamyltranspeptidase activity was reported by Hemmings et al. (2004). Groups of male Fischer 344 rats (7-9 rats/group) aged 30 days were assigned to 1 of 3 diets: 1) regular rat chow (Prolab RMH 3000); 2) rat chow with 10% Norlin (a high-ALA flaxseed cultivar); or 3) rat chow with 10% Solin (a low-ALA oilseed derived from flaxseed); both products contained 8.4 mg SDG/g flaxseed. The difference in the Norlin- and Solin-containing chows was to help differentiate between effects produced by the oil component; Norlin contains about 26.4% ALA while Solin contains only 1%. Individually housed rats in perspex cages lined with sawdust were fed the diet *ad libitum* for 100 days and body weights were recorded periodically throughout the study. On days 50 and 100, 5-7 rats[1] per group were examined, weighed, and killed by decapitation. Blood samples were taken for determination of plasma glucose and alanine aminotransferase levels and the liver and kidneys were removed, weighed, and portions homogenized for determination of protein content and γ-glutamyltranspeptidase activity.

Growth, body weights, behavior, general condition, plasma glucose and alanine aminotransferase levels, gross observation of liver and kidneys, and liver and kidney weights relative to body weight were not affected by ingestion of either type of oilseed diet. Hepatic γ-glutamyltranspeptidase activity was significantly higher in oilseed-fed (either type; approximately 2-fold higher) rats than controls. Ingestion of either type of oilseed increased the activity of γ-glutamyltranspeptidase in isolated plasma membrane fractions; Hemmings et al. (2004b) suggested that this increase is lignan-dependent and possibly linked to protection against liver injury by increasing reduced glutathione. Since the effect was similar in both types of oilseed, it was concluded that the effect was produced by the SDG rather than the ALA component. Since the only observed effect was beneficial rather than adverse, the NOAEL for both varieties of oilseed tested in this study was the single tested dietary concentration of 10%, approximately equivalent to 7500 mg/kg bw/day and including approximately 63 mg SDG/kg bw/day. The NOAEL for ALA was the level provided in the Norlin cultivar, about 2.6% dietary concentration, equivalent to 1950 mg/kg bw/day.

4.2.2.1.2. Piglet

In a study by Farmer et al. (2007) to investigate the effect of consumption of dietary flaxseed for 125 days on fatty acid profile, prepubertal development of mammary glands, and

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[1] The study authors reported that the original groups consisted of 7-9 animals, so it is unclear how they examined and decapitated 5-7 animals on day 50 and on day 100 as reported.

bone resorption, groups of Yorkshire x Landrace gilts (female piglets) aged 82 days were fed one of 4 diets: 1) basal diet[1] (control; n=14); 2) diet containing 10% flaxseed (n=13); 3) diet containing 6.5% flaxseed meal (defatted flaxseed; n=15); or 4) diet containing 3.5% flaxseed oil (n=15). Test materials were gradually added to the commercial diets over 5 days until target concentrations were reached. Animals were individually housed until the age of 151 days at which time they were allowed physical contact with a mature boar and estrous behavior was monitored. The animals received the isocaloric and isonitrogenous diets for 125 days (until 212±1 days of age). Feed consumption was recorded daily. At the age of 86, 150, and 210 days, the animals were weighed and back fat thickness was measured. Blood samples were taken from the jugular at the age of 78 and 210 days for analysis of estradiol, prolactin, and cross-linked N-telopeptides of type I collagen concentrations and to determine the plasma fatty acid profile. At 212 days of age, the gilts were euthanized, the mammary glands were removed, and extraparenchymal tissue from the cranial mammary glands was taken for assessing the fatty acid profile. Parenchymal tissue was homogenized and DNA;, dry matter, protein, and lipid content were determined. The rate of cell proliferation in the parenchyma was determined by intravenously injecting 6 gilts/group with 5-bromo-2'-deoxyuridine 2-3 hours prior to euthanasia. Slides from the parenchymal tissue were prepared for immunohistochemical staining and evaluation. Ovaries were examined from all animals and the number of corpora lutea was determined. Liver samples were taken from animals not injected with 5-bromo-2'-deoxyuridine for assessment of fatty acids.

Dietary supplementation with flaxseed products did not affect body weight, back fat thickness, number of corpora lutea, 5-bromo-2'-deoxyuridine labeling index, estrogen receptor localization, or plasma concentrations of estradiol, prolactin, or cross-linked N-telopeptides of type I collagen. Ingestion of 10% flaxseed or 3.5% flaxseed oil, but not 6.5% flaxseed meal, significantly increased the concentration of polyunsaturated fatty acids and significantly decreased the concentration of saturated and monounsaturated fatty acids in plasma and extraparenchymal tissue. The only statistically significant variable in the composition of mammary tissue was an increase in dry matter content of animals fed flaxseed compared to controls. Farmer et al. (2007) concluded that dietary flaxseed and flaxseed oil, but not defatted flaxseed meal affected fatty acid profiles; none produced any effect on mammary development or bone resorption. Since no adverse effects were seen from any of the 3 test articles, the no observed adverse effect levels (NOAELs) for flaxseed, flaxseed meal, and flaxseed oil were the single dietary concentrations tested—10% for flaxseed, 6.5% for flaxseed meal, and 3.5% for flaxseed oil.

The average feed intake of the gilts was 2.9 kg/day and their average weight was 107 kg; thus, average feed intake was 27 g/kg bw/day. Since flaxseed constituted 10% of the diet, the NOAEL was 2700 mg/kg bw/day. The NOAELs for flaxseed meal and oil were 6.5% and 3.5% of the diet, or 1750 and 950 mg/kg bw/day, respectively.

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[1] The basal diet was a commercial feed containing 52% cornmeal, 17% soybean meal, 10% ground barley, 8% canola meal, 8% wheat middlings, 2% animal and vegetable fat, 2% limestone, and <1% other components. No fish meal or other source of n-3 fatty acids was included.

4.2.2.2. Flaxseed Constituents

4.2.2.2.1. Rat

The study by Yuan et al. (1999) discussed above included an experimental group that received 3.0 mg/ml SDG in distilled water. While this treatment raised urinary excretion of lignans, no adverse effects were seen and the NOAEL was the single dose of 9 mg/kg bw/day.

Hemmings et al. (2004), also discussed above, compared the effects of two varieties of flaxseed with differing concentrations of ALA in Fischer 344 rats and found no adverse effects from either cultivar, which provided approximately 63 mg SDG/kg bw/day, which was thus the NOAEL for SDG in this study.

7-Hydroxymatairesinol (7-HMR) was evaluated in one subchronic study in the rat (Lina et al. 2005). As noted earlier, this compound is chemically related to the matairesinol found in flaxseed, the only difference between the two being the presence of a hydroxyl group rather than hydrogen at carbon 7. Six-week-old male and female Wistar outbred (Crl:WI)-(WU BR) rats were fed dietary levels of 0, 0.25, 1, and 4% (n = 20 rats/sex/level) of a potassium acetate complex of 7-HMR for 90 days. The 7-HMR was mixed into commercial powdered rodent diet (Rat & Mouse No. 3 Breeding Diet from Special Diets Services, UK), which was provided *ad libitum*. Rats of the same sex and dose group were housed in groups of 5 rats. The rats were weighed weekly and observed daily for external signs of toxicity. Food intake was measured per cage over 3- to 4-day periods, and water consumption was measured daily in weeks 1, 6, and 12. Neurobehavioral testing, consisting of detailed clinical observations outside the home cage, was conducted weekly on 10 rats/sex/group and a functional observation battery was conducted with these rats in week 13. Ophthalmoscopic examinations were conducted at baseline and during the last week of feeding on control and high-level group rats. Estrus cyclicity was evaluated from daily vaginal smears obtained from all female rats during the last 3 weeks of the study.

At necropsy, blood was collected from the abdominal aorta of 10 rats/sex/group and examined for hemoglobin, packed cell volume, red blood cell count, reticulocytes, total white blood cell count, prothrombin time, thrombocyte count, differential white blood cell count, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration. Blood collected from the tip of the tail of 10 rats/sex/group after overnight fasting in week 13 was examined for fasting glucose. Clinical chemistry analyses were performed on plasma samples derived from heparinised blood from 10 rats/sex/group. The measurements included alkaline phosphatase activity, aspartate aminotransferase activity, alanine aminotransferase activity, gamma glutamyl transferase, total protein, albumin, ratio albumin to globulin, urea, creatinine, total bilirubin, total cholesterol, triglycerides, phospholipids, calcium, sodium, potassium, chloride, and inorganic phosphate. Levels of 7-HMR, enterolactone, 7-hydroxyenterolactone, and matairesinol in plasma were measured in 10 samples/sex/group, randomly selected at the end of the study. Urine, collected from 10 rats/sex/group during the last 16 hours of a 24-hour food and water deprivation period in week 13, was analysed for volume, density, appearance, pH, glucose, occult blood, ketones, protein, bilirubin, urobilinogen, and microscopy of the sediment. Sperm samples obtained at necropsy were evaluated for epididymal sperm count, sperm motility and morphology. All animals were subjected to

complete macroscopic examination. The weights of the adrenals, brain, caecum (filled and empty), epididymes, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid (including the parathyroids), and uterus were recorded. Specimens were preserved in formalin of the aorta, axillary lymph nodes, colon, eyes, gut associated lymphoid tissue, Harderian gland, lungs, mammary gland (both genders), mesenteric lymph nodes, sciatic nerve, oesophagus, pancreas, rectum, skin, small intestine, spinal cord (at three levels), sternum with bone marrow, stomach, sublingual, and submaxillary salivary glands, trachea/bronchi, urinary bladder, Zymbals gland, and any tissue showing gross lesions. Histopathological examination was performed in all listed samples from all animals in the control and high-level group. In addition, the lungs, kidneys, liver and all gross lesions were examined from all animals of the low- and mid-level groups.

The dietary levels of 7-HMR were equivalent to average daily intakes of 0, 160, 640, and 2600 mg 7-HMR/kg bw/day, averaged across males and females, over the 90 days of the study. Dose-related increases were seen in the plasma concentrations of 7-HMR and its metabolites (chiefly enterolactone), but there were no effects on clinical signs, ophthalmoscopy, neurobehavioral observations, motor activity, or urinalysis parameters. Some reduction in feed intake and body weight gain was observed in the mid- and high-dose groups, which was ascribed to decreased palatability of the test feed. There were also isolated differences in white blood cell counts, plasma triacylglycerols, fasting blood glucose levels, and phospholipids, none of which were regarded as indicative of toxicity. No effects were observed in any of the measured sperm parameters. There was a significant increase in the mean length of the estrus cycle in high-level females. A number of statistically significant but scattered changes in absolute or relative organ weights were noted, but not regarded as toxicologically significant. The only adverse effect seen was a statistically significant and dose-dependent decrease in both absolute and relative ovary weights seen in mid-and high-dose females. Absolute ovary weights were decreased by 10%, 12%, and 15% relative to controls in the 3 test groups, while relative ovary weights were decreased by 10% and 11% in the mid- and high-level groups, respectively. No evidence of histopathology was found in any organ at any level of ingestion of 7-HMR. The authors stated that, "The minor changes in ovary weights and in estrous-cycle length without any histopathological effects in rats fed 7-HMR are not indicative of strong antiestrogenic potency." Nevertheless, based on decreased ovary weights, the authors set the NOAEL of orally administered 7-HMR in the rat at 0.25% dietary concentration, corresponding to 160 mg/kg bw/day intake, the lowest level administered.

4.2.2.2.2. Rabbit

To investigate the possible effect of lignan complex isolated from flaxseed on the hemopoietic system, rabbits were fed the complex with or without added cholesterol for 2 months (Prasad 2005). Female New Zealand white rabbits weighing 1.2-1.5 kg were divided into 4 groups (6-16 rabbits/group): 1) rabbit chow diet only; 2) rabbit chow diet supplemented with 40 mg lignan complex/kg bw/day; 3) rabbit chow diet containing 0.5% cholesterol; and 4) rabbit chow diet containing 0.5% cholesterol plus supplementation with 40 mg lignan complex/kg bw/day. The lignan complex, containing approximately 34% SDG, 15-21% cinnamic acid glucoside, and 10-11% hydroxymethyl-glutaric acid, was administered orally on

lettuce. Feed and water were provided *ad libitum*. Blood samples were taken via an ear artery before and after 1 and 2 months for testing total cholesterol, red blood cells mean corpuscular volume (MCV), red blood cells distribution width (RDW), hematocrit (Ht), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cells (WBC), granulocytes, lymphocytes, monocytes, platelets, and mean platelet volume. Although some of the initial blood values were not consistent among the groups (e.g., WBC were 4.96±0.62, 7.7±0.7, 6.34±0.33, and 6.05±0.28x10^9/l for groups 1, 2, 3, and 4, respectively), there was no indication that oral administration of 40 mg lignan complex/kg bw/day had any adverse effects on the parameters examined. The NOAEL in this study was 40 mg /kg bw/day of the lignan complex, including 14 mg SDG/kg bw/day.

4.2.2.3. Conclusions From Subchronic Toxicity Studies

Subchronic toxicity studies of flaxseed or of SDG, its primary lignan, have been conducted in the rat, rabbit, and piglet. No adverse effect were observed in any of the studies, and thus in all cases the NOAEL can be established as the highest dose tested[1]. For flaxseed itself, these doses were approximately 30,000, 7500, and 7500 mg/kg bw/day (40%, 10%, and 10% dietary concentration) in the three rat studies and 2700 mg/kg bw/day (10% dietary concentration) in the piglet study. NOAELs for SDG were 9 and 63 mg/kg bw/day in the two rat studies and 14 mg/kg bw/day in the rabbit study, the only doses tested in any of these studies. Additionally, no adverse effects were seen in a study comparing flaxseed cultivars containing high or low concentrations of ALA, and the NOAEL for ALA was 1950 mg ALA/kg bw/day, the amount ingested from the high-ALA cultivar provided at 10% dietary concentration.

4.2.3. Developmental and Reproduction Toxicity Studies

4.2.3.1. Whole Flaxseed

To evaluate the potential reproductive and developmental effects of whole flaxseed, Tou and Thompson (1997; a poster presentation available only as an abstract) fed rats (number and strain not reported in the poster) a basal diet (not described in the poster) or the same basal diet supplemented with either 5 or 10% flaxseed during one of 5 periods: a) pregnancy only, b) both pregnancy and lactation, c) from weaning to 50 days of age, d) from weaning to 132 days of age (adulthood), or e) dam's pregnancy and pups adulthood.

No consistent adverse effects were seen at the 5% concentration of flaxseed. When 10% flaxseed was fed during pregnancy, there was no effect on gestation length, parturition, litter size, or survival, but pups showed statistically significantly decreased birth weights. When 10% flaxseed was fed during both pregnancy and lactation, weight gain was significantly slowed, the anogenital distance was significantly shortened, and the absolute ovarian weight of the pups was statistically significantly increased. Flaxseed administration after weaning had no effect on onset

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[1] The NOAEL in Lina et al. (2005) was not the highest dose tested, but the test article in that study was 7-HMR, which, while chemically related to the matairesinol found in flaxseed, is itself only a minor flaxseed constituent.

of puberty in females, but absolute ovarian weight was significantly increased at 50 days; at 132 days premature vaginal opening and significantly increased absolute ovarian weight was seen in females while significantly increased weight of prostate, seminal vesicles, accessory sex glands, and testes were observed in males. The authors concluded that a 10% concentration of flaxseed in the diet (approximately 7500 mg/kg bw/day) "can cause hormonally related effects which depended on timing of the exposure," but that no adverse effects were seen when flaxseed constituted only 5% of the diet, equivalent to about 3750 mg/kg bw/day, and this may be considered the NOAEL.

The possible effect of dietary flaxseed administration on estrous cycling was studied in rats by Orcheson et al. (1998). Groups of individually housed female Sprague-Dawley rats aged 58 days (6 rats/group) were randomly allocated into 1 of 8 treatments: 1) a high-fat basal diet based on the AIN-76A diet (negative control); 2) basal diet plus daily gavage with 1.0 ml of 0.75 mg SDG/ml in distilled water; 3) basal diet plus daily gavage with 1.0 ml of 1.5 mg SDG/ml in distilled water; 4) basal diet plus daily gavage with 1.0 ml of 3.0 mg SDG/ml in distilled water; 5) basal diet containing 2.5% flaxseed; 6) basal diet containing 5% flaxseed; 7) basal diet containing 10% flaxseed; or 8) basal diet with daily gavage of 1 mg tamoxifen/kg bw in 1 ml corn oil (positive control). Groups 1 and 5-7 also received daily gavage of distilled water. Animals were monitored daily for estrous cycling by means of vaginal smears 2 weeks prior to treatment and during the 4 weeks of treatment. Body weights were recorded weekly, feed intake was measured every 2 days, and urine was collected daily after 1 week of treatment. At the end of the study, rats were asphyxiated by CO_2 inhalation, gross pathology was performed, and organ weights were recorded. Urine was pooled into 3-day samples for each rat and analyzed for enterodiol, enterolactone, and secoisolariciresinol using GC-MS.

There was an apparent but non-statistically significant dose-related increase in the number of rats with either acyclic (no longer cycling) or irregular (>10 days in length) estrous cycles on the flaxseed diet or oral SDG. In the groups with the highest dose of flaxseed and SDG, 66% of the rats were either acyclic or irregular compared to approximately 16% in the basal diet group. Eighty-three percent of tamoxifen-treated rats were no longer cycling ($P<0.003$ vs. controls). Of the rats still cycling before and after treatment, rats on flaxseed and SDG treatments tended to have longer cycles, but this effect was only significantly different from controls for the 5% flaxseed group and was not dose-dependent. Urinary excretion of lignans was significantly higher in flaxseed groups than corresponding SDG groups ($P<0.05$). Rats fed flaxseed or orally administered SDG showed no differences from basal diet controls in body weight, feed intake, organ weights (ovaries, liver, heart, kidneys, spleen, cecum, and colon), or gross pathology. Administration of flaxseed or SDG was well tolerated and produced no toxicity in rats at the levels tested, equivalent to 1900, 3750, and 7500 mg/kg bw/day of flaxseed. The NOAEL for this study was 7500 mg flaxseed/kg bw/day, the highest dose tested/

Tou et al. (1998a; orally presented at a conference and available only in abstract form) further evaluated the reproductive effects of flaxseed in pregnant rat dams (number and strain not reported) that were fed basal diet or basal diet supplemented with 5 or 10% flaxseed, approximately equivalent to 3750 and 7500 mg/kg bw/day, during pregnancy and lactation. After

weaning, the pups of dams that had been fed flaxseed were fed only basal diet, while the offspring of basal-diet-fed dams were given either 5 or 10% flaxseed-supplemented diets.

No effects were seen due to flaxseed exposure at either 5 or 10% of the diet fed after weaning, but perinatal exposure resulted in several effects that were dose-dependent. Some of the effects appeared to be paradoxical. While male rats receiving 5% flaxseed diets exhibited signficantly decreased absolute prostate weights, those receiving 10% flaxseed had significantly increased absolute prostate weights. Similarly, 5% flaxseed significantly elevated the estradiol/testosterone ratio, but 10% significantly increased both estradiol and testosterone. Females receiving a 5% flaxseed diet exhibited delayed puberty and persistent diestrus (with no change in sex hormone levels), while those receiving 10% flaxseed had premature and irregular cycles. The authors concluded that flaxseed exposure during pregnancy and lactation, but not after weaning, can influence the reproductive development of rats.

Tou et al. (1998b) fed groups of individually housed pregnant Sprague-Dawley rats (7 rats/group) either basal diet (AIN-93), basal diet plus 5% flaxseed, basal diet plus 10% flaxseed, or basal diet with a daily gavage of 1.5 mg SDG dissolved in 1 ml distilled water throughout gestation. Rats not receiving SDG were gavaged daily with 1 ml distilled water. Maternal weight gain and feed intake were recorded daily during gestation and every 2nd day during lactation; offspring weight also was recorded. Litter size, birth weight, stillbirth incidence, postnatal pup survival, and percentage of females were recorded. Dams continued on their respective diets throughout lactation; on postnatal day 21 dams and 2 offspring (1/sex) from each litter were killed by CO_2 asphyxiation. Major organs, particularly reproductive organs, were removed and weighed. Surviving offspring were switched to basal diet and selected offspring (6-10/sex/group) were killed by CO_2 asphyxiation on postnatal day 50 or 132. Offspring weight gain was recorded every 2nd day and anogenital distance was measured on postnatal days 3 and 21. Puberty onset was determined in females by daily monitoring for the opening of the vaginal aperture. During postnatal days 40-50 and 100-132, daily vaginal smears were taken to microscopically determine the stage of estrous cycle. One cycle was considered the number of days to complete all 4 stages and rats were considered acyclic if they remained at one stage for more than 10 days. Prostates from males were histologically examined. To test for lignan transfer through lactation, 3 dams receiving SDG were gavaged with 1.5 mg ^{3}H-labeled SDG (instead of unlabeled SDG) on postnatal day 20. Two offspring (1/sex) were allowed to remain with the dams; after 24 hours the dams and offspring were killed by CO_2 asphyxiation, and blood, all tissues, and gastrointestinal contents were collected for analysis of radioactivity.

Administration of 5 or 10% flaxseed or SDG had no effect on maternal feed intake, maternal weight gain, gestation length, litter size, live birth index, postnatal pup survival, or number of female offspring when compared with control animals. Significantly lower birth weights were observed in offspring from dams fed 10% flaxseed, but not the other treatments. When compared to basal diet administration, 10% dietary flaxseed significantly reduced the body weight gain of male offspring, but not females, after lactation. The difference in anogenital distance between postnatal day 3 and 21 also was significantly less in both sexes from this treatment group than among controls. Puberty onset was significantly delayed in females from the 5% flaxseed (34.9±0.6 days) or SDG (34.7±0.7 days) groups, but was significantly earlier in

females fed 10% flaxseed (25.5±0.3 days) when compared to controls (30.1±0.8 days). Estrous cycling was significantly lengthened in females fed 10% flaxseed during postnatal days 40-50 and 100-132. During postnatal days 100-132, 1 of 6 rats fed 5% flaxseed, 1 of 7 rats administered SDG, and 2 of 10 rats fed 10% flaxseed were acyclic. In female offspring, the uterus-to-body weight ratio was significantly increased at postnatal day 21 in all treatment groups compared to controls; however, at postnatal days 50 and 132, there were no differences from controls. Ovaries-to-body weight ratio was significantly decreased at postnatal day 21 in rats fed 5% flaxseed or administered SDG, but not in those fed 10% flaxseed. At postnatal days 50 and 132, ovaries-to-body weight ratios were significantly increased only in rats fed 10% flaxseed. In males at postnatal day 132, sex glands and prostate weights relative to body weight were significantly increased in rats fed 10% flaxseed. The other major organs of the offspring were not affected by treatment. Neither males nor females from the treatment groups showed any differences in body weight when compared with control values. Extensive cell proliferation—increased amounts of secretory epithelial cells forming more papillary infoldings and secondary projections—was noted in the ventral lobe of the prostate of males fed 10% flaxseed. Radioactivity detected in the blood and tissues of offspring nursing from dams administered ^{3}H-labeled SDG indicated that lignans were transferred via milk. The authors concluded that 5 or 10% flaxseed or SDG produced no adverse effects on pregnancy other than lowered birth weights at 10% flaxseed, but hormone-related effects on offspring (e.g., prostrate growth, timing of pubery onset, estral acyclicity) may affect reproduction.

A study was conducted by Tou and Thompson (1999) to assess any potential effect of flaxseed or its components on mammary gland structures. Twenty-eight 77-day-old pregnant Sprague-Dawley rats were divided into 5 groups and fed basal diet (semi-purified AIN-93G), basal diet plus 5% flaxseed, basal diet plus 10% flaxseed, basal diet plus 1.82% flaxseed oil, or basal diet plus a daily gavage of 1.5 mg SDG dissolved in 1 ml distilled water throughout gestation and lactation. Animals not receiving SDG also were gavaged daily with 1 ml distilled water. Following weaning (postnatal day 21), female offspring were individually housed and subdivided into various exposure scenarios within each treatment group. Some control rats remained on their dam's basal diet and remained controls. Female offspring that were continued on their dam's flaxseed diet represented lifetime exposure and those that originally were on basal diet and were switched to a flaxseed diet represented exposure after weaning. To represent exposure during gestation and lactation, female offspring from dams on the flaxseed diet were switched to basal diet for postnatal days 21-50. On postnatal day 50 (puberty), 6 offspring/group were killed by CO_2 asphyxiation and whole mounts of mammary gland were prepared for counting of mammary structures. Puberty onset was determined in females by daily monitoring for the opening of the vaginal aperture. Up to postnatal day 50, daily vaginal smears were taken to microscopically determine the stage of estrous cycle. One cycle was considered the number of days to complete all 4 stages with a normal cycle being 4-5 days (1-2 days of estrus). Rats were considered acyclic if they remained at one stage for more than 10 days and cycles were considered prolonged if any stage was >3 days. At termination, ovaries were removed and weighed. Blood samples also were collected for determination of serum estradiol.

The development of mammary gland structures was altered (as indicated by a statistically significant decrease in terminal end buds and a statistically significant increase in alveolar buds)

by 10% dietary flaxseed fed during gestation and lactation and over a lifetime, but not when fed only after weaning. Similarly, feeding 5% flaxseed during gestation and lactation and over a lifetime, but not after weaning, also altered the development of mammary gland structures (as indicated by a statistically significant decrease in terminal end buds). Oral administration of SDG during gestation and lactation had the same effect, while dietary flaxseed oil had no effect. Exposure to either concentration of dietary flaxseed after weaning had no effect on puberty onset, estrous cycling, ovarian weight relative to body weight, or serum estradiol; however, when compared to controls exposure to 10% flaxseed during gestation and lactation or over a lifetime significantly lengthened the estrous cycle (by approximately 2 days), shortened the time to puberty onset (by approximately 5 days), increased relative ovarian weight, and increased serum estradiol levels. Exposure to 5% flaxseed or SDG during gestation and lactation or over a lifetime (i.e., based on offspring that were continued on their dams' flaxseed diets) significantly lengthened the time to onset of puberty (by approximately 4-5 days) when compared to controls but had no effect on estrous cycle length. Dietary flaxseed oil did not affect puberty onset or estrous cycle length. Relative ovarian weight and serum estradiol were not affected by 5% flaxseed, SDG, or flaxseed oil. The authors concluded that dietary flaxseed produces changes in mammary structures when exposure begins through gestation. The dietary concentration of flaxseed (5 vs. 10%) appears to produce different endocrine changes. The lower concentration produced antiestrogenic effects, whereas the higher concentration produced estrogenic effects. Tou and Thompson (1999) suggested that the reduction in terminal end bud density, which was seen at both 5% and 10% dietary concentration of flaxseed, indicates potential protection against mammary cancer.

Reproductive indices and sex hormone levels were studied in pregnant Sprague-Dawley rats fed various concentrations of dietary flaxseed (Tou et al. 1999). Pregnant rats (5 rats/group) were individually housed in polycarbonate cages and fed phytoestrogen-free basal diets (AIN-93) containing 0 (control), 5, or 10% flaxseed *ad libitum* throughout gestation and lactation. Body weights of dams and offspring and feed intake were recorded every 2nd day. On postnatal day 21 (end of lactation period), 1 offspring/sex/litter was killed by CO_2 asphyxiation and the ovaries, uterus, testes, and accessory sex glands were examined and serum estradiol and/or testosterone was determined. Surviving offspring of control dams were continued on basal diet alone or were fed 5 or 10% flaxseed until postnatal day 50 (puberty) or 132 (adulthood). The surviving offspring from dams fed flaxseed were continued on their dam's diet. At postnatal day 50 or 132, randomly selected groups of male (n=5-7) and female (n=5-6) offspring from each dam in each treatment group were killed by CO_2 asphyxiation. Females were terminated in the same phase of estrous cycle as determined by vaginal smears. Major and reproductive organs were removed and weighed. Blood samples were taken for analysis of estradiol or testosterone levels. Puberty onset was determined in females by daily monitoring for the opening of the vaginal aperture. Up to postnatal day 50 and during postnatal days 100-132, daily vaginal smears were taken to determine the stage of estrous cycle. One cycle was considered the number of days to complete all 4 stages with a normal cycle being 4-5 days (1-2 days of estrus). Rats were considered acyclic if they remained at one stage for more than 10 days and cycles were considered prolonged if any stage was >3 days. Prostates from male offspring were examined histopathologically.

Puberty onset was significantly earlier and occurred at a significantly reduced body weight than controls in rats having a lifetime exposure (i.e., offspring of dams receiving flaxseed that were continued on flaxseed to postnatal day 132) to 10% flaxseed, whereas, lifetime exposure to 5% flaxseed caused a significant delay in puberty onset at a significantly increased body weight when compared to controls. Length of estrous cycling was significantly increased over controls at postnatal days 50 and 132 in rats with a lifetime exposure to 10% flaxseed, but not 5% flaxseed. Similarly, serum estradiol levels were significantly increased over control values at postnatal days 50 and 132 in female rats with a lifetime exposure to 10% flaxseed, but not 5% flaxseed. Exposure to 5 or 10% flaxseed after weaning had no effect on puberty onset, weight at onset, estrous cycling, or serum estradiol levels. In male offspring fed 10% flaxseed but not 5% flaxseed for a lifetime, serum testosterone and estradiol were significantly increased over control values. Dietary flaxseed had no effect on testosterone or estradiol when fed after weaning. With the exception of reproductive organs, the major organ weights of flaxseed-fed rats were no different from controls. Exposure to dietary flaxseed after weaning produced no changes in ovarian, accessory sex gland, prostate, seminal vesicle, or testicular weights. Lifetime exposure did show some statistically significant changes in organ weights relative to body weight when compared to control values: increased ovarian weights on postnatal days 50 and 132 in females fed 10% flaxseed, increased accessory sex gland, seminal vesicle and testicular weights on postnatal day 132 in males fed 10% flaxseed, and increased prostate weight on postnatal day 132 in males fed 5 or 10% flaxseed. Histopathology of the prostate from males fed 10% flaxseed over a lifetime showed extensive epithelial cell proliferation. Tou et al. (1999) concluded that dose, timing, and duration of flaxseed exposure affect reproductive indices and sex hormone levels in growing rats.

A study was conducted in Sprague-Dawley rats to assess the potential for flaxseed or flaxseed meal to affect spermatogenic or endocrine function in male rats (Sprando et al. 2000a). Pregnant female rats (number/group not stated) were randomly assigned to 1 of 5 diets and fed those diets from gestation day 0 to the end of weaning of the F1 generation: 1) basal AIN-93 diet (control); 2) basal diet with 20% flaxseed plus soybean oil and flaxseed oil; 3) basal diet with 13% flaxseed meal plus soybean oil and a small amount of flaxseed oil; 4) basal diet with 40% flaxseed with no soybean oil but all the fat derived from flaxseed; or 5) basal diet with 26% flaxseed meal plus soybean oil and a small amount of flaxseed oil. After weaning (postpartum day 21), 1-2 males/litter were randomly selected and maintained on the same diets as their dams for 70 days (17-19 F1 males/group). After 70 days of treatment, the F1 males were killed and assessed for various reproductive parameters, including qualitative analysis of gonadal tissues, testicular spermatid and epididymal sperm counts, cauda epididymal sperm morphology, and serum concentrations of testosterone, luteinizing hormone, and follicle stimulating hormone. The epididymides, heart, spleen, liver, kidneys, adrenals, seminal vesicles, and prostate were removed and their weights recorded.

Dietary administration of flaxseed or flaxseed meal did not affect terminal body weights, testes, epididymides, or seminal vesicle weights, mean spermatid counts, mean number of sperm abnormalities, daily sperm production rates, seminiferous tubule fluid testosterone concentrations, or serum follicle stimulating hormone when compared with rats fed the control diet. Prostate weights were significantly lower than controls in rats fed 13 or 26% flaxseed meal

or 20% flaxseed. Other statistically significant findings when compared with corresponding controls included: increased serum testosterone in rats fed 26% flaxseed meal, increased serum luteinizing hormone in rats fed 20 or 40% flaxseed or 26% flaxseed meal, increased cauda epididymal weight in rats fed 20 or 40% flaxseed, increased cauda epididymal sperm count in rats fed 20 or 40% flaxseed or 26% flaxseed meal, decreased absolute kidney and liver weights in rats fed any flaxseed product, and increased absolute heart weight in rats fed 20% flaxseed or 26% flaxseed meal. The authors concluded that dietary flaxseed or flaxseed meal at these high doses (equivalent to 15,000 and 30,000 mg/kg bw/day of flaxseed) had some effect on the male rat reproductive system; however, histological effects on spermatogenesis were not observed in any of the flaxseed- or flaxmeal-treated groups.

Sprando et al. (2000b) further assessed the potential of flaxseed to affect spermatogenesis in a second experiment in which pregnant Sprague-Dawley rats were randomly assigned to the 5 dietary regimens described above (Sprando et a. 2000a) and maintained on the diets until the end of weaning of the F1 generation offspring. Litters were culled to 10 pups, 5 males and 5 females, on postnatal day 4. On postnatal day 21, 1-2 male pups/litter from as many litters as possible were selected to represent the F1 generation, and were retained on the same diets as their dams for 70 days. The male rats were then anesthetized with CO_2, the heart and internal organs were exposed, and the right testis was perfusion-fixed. The volume density of the testicular components, including seminiferous tubules, tubular lumen, interstitium, Leydig cells, blood vessels, and macrophages, was measured.

No significant differences were found in body weights or testis weights in any of the groups. The volumes of the seminiferous tubules in the rats receiving 20% and 40% flaxseed were lower than in the controls, but significant differences were not observed in Sertoli cell nucleolar numbers, nor in the length or surface areas of the seminiferous tubules. The authors concluded that flaxseed and flaxmeal did not adversely affect spermatogenic activity in the treated animals and the statistically significant differences in volume were not biologically significant and were most likely due to sampling error. They further concluded that the information obtained in this study confirms and extends the findings of Sprando et al. (2001a) and suggests that flaxseed and flaxmeal do not affect spermatogenesis or testis structure in the rat at the doses tested in this study, approximately equivalent to 9750 and 19,500 mg/kg bw/day of flaxseed meal and 15,000 and 30,000 mg/kg bw/day of flaxseed.

To examine the possible effect of flaxseed or its purified lignan SDG on reproductive indices, young rats were exposed to dietary flaxseed or SDG through lactation or continuously through to adulthood (Ward et al. 2001a). Twenty Sprague-Dawley rats that had just delivered litters of pups were randomly assigned to 1 of 3 diets: 1) basal diet (AIN-93G); 2) basal diet plus 10% flaxseed (equivalent to 7500 mg/kg bw/day); or 3) basal diet plus 1.8 mg SDG/kg bw/day. Dams were individually caged and maintained on these diets during lactation until the pups were weaned. Feed intake of dams was recorded every 2 days throughout lactation. After weaning, pups (23-44 males/group and 22-47 females/group) were either maintained on the same diet as their dams or were switched to basal diet until postnatal day 50 (adolescence) or 132 (adulthood). Pup body weights were recorded weekly and feed intake was monitored. Water and diet were provided *ad libitum*. Rats were killed by CO_2 asphyxiation at weaning (dams), on postnatal day

50 (pups), or postnatal day 132 (pups). Anogenital distance was measured at postnatal days 2 and 21. Puberty onset was determined in females by daily monitoring for the opening of the vaginal aperture. During postnatal days 100-132, daily vaginal smears were taken to microscopically determine the stage of estrous cycle. One cycle was considered the number of days to complete all 4 stages, proestrus, estrus, metestrus, and diestrus. At termination, reproductive organs were removed, weighed, and subjected to histological examination.

Dietary administration of 10% flaxseed or its purified lignan to rats during suckling or continuously to adolescence or adulthood failed to produce any adverse effect in any of the parameters examined including anogenital distance from birth to postnatal day 21, age and body weight at female puberty onset, estrous cycle length, reproductive organ weights at postnatal days 50 and 132, and the histology of the reproductive organs—uterus, ovaries, and prostate. Since intakes of 7500 mg/kg bw/day of flaxseed or of 1.8 mg/kg bw/day were without harm, The authors concluded that these findings provide evidence that exposure to levels of flaxseed or its lignans attainable in the human diet appears to be safe with respect to developing offspring.

Using a design and experimental diets identical to those used in in the study described above (Ward et al. 2001a), Ward et al. (2001b) investigated the effect of exposure to flaxseed or SDG on bone strength of male rats. Twenty pregnant Sprague-Dawley rats were fed basal diet (AIN-93G) from gestation day 2 until delivery on gestation day 21 or 22. Prior to lactation they were randomized to basal diet, basal diet + 10% flaxseed, or basal diet + 293 μmol SDG/kg diet, equivalent to the SDG in the 10% flaxseed diet. Offspring were weaned at postnatal day 21 and either continued on their mother's diet or on basal diet. Rats were killed on postnatal day 50 or 132 (representing adolescence or young adulthood) and their femurs excised for measurement of weight, length, and width. To assess bone quantity, bone mineral content (BMC) and bond mineral density (BMD) of femurs were measured by dual-energy x-ray absorptiometry. The mechanical strength of femurs was determined by 3-point bending.

At post-natal day 50, measures of bone strength were reduced among rats receiving the 10% flaxseed diet, but there were no differences in bone size, BMC, or BMD. No effect was seen in animals receiving SDG. However, at day 132 there were no significant differences between any of the groups with regard to bone size, strength, or mineralization. The authors concluded that, "exposing male rats to a diet containing 10% flaxseed or an equivalent quantity of lignan either during lactation only or through to early adulthood is safe with respect to bone health."

Cesarean-derived, viral antibody-free rats were used in a 2-phase study conducted under Good Laboratory Practice (GLP) to examine the possible effects of ground flaxseed or flaxseed meal on development and reproduction (Collins et al. 2003). Pregnant rats were housed individually in stainless steel hanging cages and divided into 5 treatment groups (37-38 rats/group): 1) AIN-93 diet only (control); 2) AIN-93 diet containing 20% ground flaxseed; 3) AIN-93 diet containing 13% flaxseed meal (defatted flaxseed); 4) AIN-93 diet containing 40% ground flaxseed; and 5) AIN-93 diet containing 26% flaxseed meal. The rats were fed these diets and given water *ad libitum* during the entire study period.

For the first phase, teratology, 22-23 pregnant rats/group were examined and then euthanized with CO_2 on gestation day 20. Euthanized rats underwent necropsy including removal and weighing of liver, kidneys, heart, brain, spleen, ovaries, and adrenal glands. Tissue samples were preserved for histopathological evaluation. Before removing any fetuses, the uterus was examined in situ for any resorptions or implantations and the number of live or dead fetuses. In the instances where the rat did not appear pregnant, the uterus was stained with ammonium sulfide to enhance resorption sites. All fetuses and both ovaries were removed. The ovaries were examined under a lighted magnifying glass and the number of corpora lutea was counted. Viable fetuses were removed and examined for uterine position, sex, weight, crown-rump length, anogenital distance, and any abnormalities before being euthanized and fixed in 95% ethanol for evaluation of skeletal abnormalities or Bouin's solution for evaluation of soft tissue abnormalities. Skeletal evaluations included examination of sternebrae, ribs, vertebrae, dorsal arches, skull, and appendicular skeleton.

The surviving pregnant rats (15 rats/group) were allowed to complete gestation and care for their young as part of the second phase of the study. Dams were observed daily during postnatal days 0-21 and litters containing more than 10 pups were culled to 10 pups on postnatal day 4. On postnatal days 0, 7, 14, and 21, pups were weighed and sexed and their anogenital distance was measured. On postnatal days 0, 4, 7, 14, and 21, reproduction, survival, and anogenital distance divided by body weight were calculated. On postnatal day 21, 2 subsets of pups were randomly selected (1 pup/sex/subset): one subset for euthanasia and necropsy and one subset for observation of sexual development to postnatal day 90. Males were observed for development of reproductive organs and endocrine functions. Females were observed for vaginal opening and their estrous cycle was monitored daily during postnatal days 70-90. At necropsy, the liver, kidneys, brain, spleen, ovaries and adrenal glands were removed and weighed from randomly selected dams on postnatal day 21 and F1 females on postnatal day 90 and the thymus, liver, spleen, and brain were removed and weighed from pups terminated on postnatal day 21. Selected tissues from both phases of the study were microscopically examined and histopathological examination focused on tissues from reproductive organs and mammary glands.

Feeding ground flaxseed or flaxseed meal was well tolerated and weight gain was similar among treated and control animals; however, one rat from the 40% ground flaxseed group died close to term (cause of death not stated, but not believed to be test-article related) and all its fetuses were dead. Feed consumption was statistically significantly reduced in rats fed 26% flaxseed meal during the teratology phase. No other significant changes in feed or water consumption were noted in either the teratology or reproduction phase. There was >90% fertility in all treatment groups. Gestation length was not affected by treatment with ground flaxseed but was slightly decreased in the flaxseed meal groups (control, 22.0±0.1 days; 13% flaxseed meal, 21.6±0.1 days; 26% flaxseed meal, 21.6±0.1) with the decrease reaching statistical significance at the highest concentration when compared to controls. All the parameters assessed in the teratology phase were similar between treated and control animals. Fetal bone and soft tissue development were not affected by treatment with ground flaxseed or flaxseed meal.

Survival of F1 rats to postnatal day 21 was similar between control and treated groups. At postnatal day 21, the anogenital distance was significantly increased ($p < 0.01$) in F1 females but not males from dams fed 20 or 40% ground flaxseed when compared with controls. Flaxseed meal did not affect the anogenital distance. Puberty as indicated by preputial separation was significantly delayed ($p < 0.01$) in F1 males from dams fed 13 or 26% flaxseed meal but not ground flaxseed when compared with controls. There was a statistically significant decrease in the number of F1 females with regular estrous cycles (20 and 40% ground flaxseed and 26% flaxseed meal groups) and a statistically significant increase in the number of F1 females with highly irregular estrous cycles (26% flaxseed meal group) when compared with controls. At weaning, body weight was significantly lower in F1 females from dams fed either concentration of ground flaxseed or flaxseed meal compared to controls. Liver and thymus weight to body weight or brain weight ratios were significantly decreased in weanling F1 females from dams fed either concentration of ground flaxseed or flaxseed meal. Weanling F1 males from any flaxseed group had body weights similar to controls. Thymus weight to body weight ratios were significantly decreased in weanling F1 males from dams fed 40% ground flaxseed and thymus to brain weight ratios were significantly decreased in weanling F1 males from dams fed 20 or 40% ground flaxseed or 26% flaxseed meal when compared with controls. Liver to body weight or brain weight ratios were significantly decreased in weanling F1 males from dams fed 26% flaxseed meal compared to controls.

F1 females from dams fed 20 or 40% ground flaxseed or 26% flaxseed meal gained significantly more weight than controls during postnatal days 21-90. By postnatal day 90, treated F1 female body weights were similar to controls except for F1 females from dams fed 26% flaxseed meal which had significantly higher body weights compared to controls. Also at postnatal day 90, kidney to body weight ratios were significantly lower than controls in F1 females from dams fed either concentration of ground flaxseed or flaxseed meal. Kidney to brain weight ratios and other organ to body/brain weight ratios were not affected. The authors concluded that ingestion of up to 40% ground flaxseed (approximately 30,000 mg/kg bw/day) or 26% flaxseed meal (approximately 19,500 mg/kg bw/day) during gestation had no effect on fertility or fetal development; however, continued ingestion post partum, during lactation, and post lactation showed some hormone-related effects on postnatal development and sexual maturation.

In a parallel study to the Collins et al. (2003) study described above, rat embryos allowed to gestate to day 9 were cultured in serum derived from blood samples taken from 10 randomly selected pregnant rats of each treatment group in the Collins et al. (2003) study at the time of Cesarean section (Flynn et al. 2003). The embryos were cultured for 45 hours, removed from the culture medium, washed, and evaluated for viability and presence of any trauma, such as torn yolk sacs, unrelated to treatment. Viable embryos without trauma were fixed in neutral formalin, measured, and evaluated for any abnormalities. All embryos from all treatment groups were viable. The only statistically significant effect on overall growth and development was reduced crown-rump length in embryos cultured in serum from dams fed 20% ground flaxseed when compared to controls. In terms of abnormalities, the incidence of heart looping (abnormal development of left-right asymmetry) was significantly higher in embryos cultured in serum from dams fed 20% ground flaxseed or 26% flaxseed meal and the incidence of flexion defects

was significantly higher in embryos cultured in serum from dams fed 20% ground flaxseed when compared with controls. The findings indicate that diets supplemented with 15,000 or 30,000 mg/kg bw/day of ground flaxseed or 97500 or 19,500 mg/kg bw/day of flaxseed meal do not produce serum factors that are embryotoxic. The authors concluded that "serum from rats exposed to high levels of flaxseed or flaxseed meal is not directly embryotoxic to organogenesis-staged rat embryos in a dose-related manner, suggesting that such expsoures are not teratogenic." They further noted that "This finding is consistent with observations from a concurrent *in vivo* reproductive toxicology study [Collines et al. (2003)] in which no frank terata were observed in the offspring from dams fed high levels of flaxseed."

In a companion study to the Collins et al. (2003) and Flynn et al. (2003) studies, Wiesenfeld et al. (2003) evaluated the nutritional aspects of dietary flaxseed or flaxseed meal. Groups of pregnant Sprague-Dawley rats were individually housed in stainless steel suspended cages and given 1 of the 5 diets previously described (Collins et al. 2003). On gestation day 20, 10 pregnant rats per group were euthanized and another 10 pregnant rats per group continued their diet regime throughout gestation (21-23 days). Ten male pups from each of these dams were allowed to stay with their dams until weaning (postnatal day 21-23). In litters with more than 10 pups, pups were randomly culled at postnatal day 4 and used for analysis of stomach lipid content. Blood samples were taken for clinical chemistry analysis including blood urea nitrogen, albumin, iron, calcium, total protein, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, triacylglycerols, and total and HDL cholesterol. Serum and tissues were analyzed for lipids and vitamins A and E. Prostaglandin E_2 was measured in serum and liver samples.

Flaxseed and flaxseed meal supplementation had no effect on body weights, absolute organ weights, or growth of dams and offspring. Both concentrations of flaxseed meal produced a significant decrease in the relative (to body weight) liver weight of dams compared to controls. Relative heart weight of rats fed 20% flaxseed or 26% flaxseed meal was significantly increased over controls. Relative organ weights of the offspring were not affected by treatment. Dietary flaxseed and flaxseed meal both changed fatty acid profiles, flaxseed to a greater extent. In serum, "gastric milk," and liver of dams and offspring fed flaxseed or flaxseed meal, ALA and eicosapentaenoic acid levels increased, linoleic and arachidonic acid levels decreased, and docosahexaenoic acid was unchanged. Serum and liver prostaglandin E_2 in dams and offspring were not affected by dietary flaxseed or flaxseed meal when compared with control values. Some statistically significant changes in clinical chemistry parameters of 90-day post weaning rats compared to controls were noted: reduced total and HDL cholesterol in rats fed 40% flaxseed; reduced ratio of total cholesterol/HDL ratio in rats fed 13 or 26% flaxseed meal; reduced serum calcium in rats fed 20% flaxseed, 13% flaxseed meal, or 26% flaxseed meal; increased alanine amino transferase in rats fed 40% flaxseed; reduced serum protein and creatinine in rats fed 40% flaxseed; and reduced serum protein in rats fed 13 or 26% flaxseed meal. Liver vitamin E levels were significantly lower than controls in offspring fed 40% flaxseed, although serum vitamin E levels were not affected. The authors concluded that, while there were differences in fatty acid absorption and metabolism between dams and offspring fed dietary flaxseed or flaxseed meal, there were no toxic effects associated with feeding flaxseed or

flaxseed meal at levels as high as 9,950 or 19,500 mg/kg bw/day of flaxseed meal or 15,000 or 30,000 mg/kg bw/day of flaxseed.

The developmental effects of flaxseed were further evaluated by Hemmings and Barker (2004). Starting on gestation day 18, pregnant Fischer 344 rats (number of animals not stated) maintained in individual perspex cages lined with sawdust were fed either regular rat chow diet (Prolab RMH 3000 from PMI Nutrition International) or 10% flaxseed chow and allowed to give birth and rear their young. Pups were weaned on postnatal day 31 and maintained on the same diet as their dams. Developmental and behavioral tests were conducted on the pups and growth curves were plotted. On postnatal days 10, 28, 48, and 86, selected pups from both groups were evaluated, weighed, and killed by decapitation. Following decapitation, blood samples were collected and liver and kidneys were removed and weighed. Plasma was analyzed for glucose, alanine aminotransferase, and γ-glutamyltranspeptidase levels. Liver protein content and γ-glutamyltranspeptidase activity also was determined.

Growth curves and developmental and behavioral indices were similar between the treated and control groups. Plasma glucose, alanine aminotransferase, and γ-glutamyltranspeptidase levels in treated animals showed no differences from controls. Gross examination and organ-to-body weight ratios of the liver and kidneys also showed no differences from controls. γ-Glutamyltranspeptidase activity in the liver of flax-fed animals was greater than that of controls throughout the study and reached statistical significance at adulthood. The sex-dependent differences in the γ-glutamyltranspeptidase activity were markedly different between flax-fed and control animals. The authors suggested that this implicates an estrogenic effect of a flaxseed lignan and concluded that dietary flaxseed has no effect on growth, development, or behavior at the dose tested (equivalent to approximately 7,500 mg/kg bw/day), but may have a hepatoprotective effect.

4.2.3.2. Flaxseed Constituents

Five of the studies discussed above included doses of SDG alone—Orcheson et al. (1998), Tou et al. (1998b), Tou and Thompson (1999), Ward et al. (2001a), and Ward et al. (2001b). In none of these studies were any effects produced by SDG alone that were not also observed in rats receiving flaxseed providing the same level of SDG. Although a number of observations were consistent with an estrogenic or antiestrogenic effect of high doses of flaxseed, most likely due to the lignan content, these effects were not regarded as necessarily adverse. Additionally, Tou and Thompson 1999 included a group of rats that received a diet containing 1.82% flaxseed oil; this treatment produced no adverse effects on any parameter evaluated.

Prenatal developmental toxicity of the lignan 7-hydroxymetairesinol (7-HMR) was investigated by Wolterbeek et al. (2004). As noted previously, this substance is a minor constituent of flaxseeds, but differs from the major lignan metairesinol only in the replacement of one hydrogen on carbon 7 with a hydroxyl group and can also serve as a precursor to the mammalian lignan enterolactone. Mated female Wistar rats were fed diets with 0, 0.25, 1, or 4% potassium acetate complex of 7-HMR for days 0-21 of gestation. The test doses were

approximately 0, 0.16, 0.60, and 2.06 g/kg bw/day, respectively. After sacrifice on day 21, the rats were examined for the following parameters of reproductive performance—fecundity index, gestation index, number of corpora lutea, number of implantations, pre- and post-implantation loss, number of early and late resorptions, number of live and dead fetuses, sex-ratio, and weight of reproductive organs. The fetuses were examined for external, visceral, and skeletal changes.

No effects were seen on reproductive performance nor were any treatment-related findings noted following examination of the fetuses. However, half of the mated dams in the high-dose group failed to thrive due to large decreases in their feed intake and were sacrificed early, and body weights of the remaining members of the high-dose group were reduced. The NOAEL for maternal effects was 1% (the mid level) and the NOAEL for fetal development throughout gestation was 4%, the highest concentration tested.

In a study investigating the possible effects of flaxseed on bone development (Cohen and Ward 2005), groups of male (n=14-22 mice/group) and female (n=10-19 mice/group) weanling 129 Sv/Ev mice were individually housed and fed 10% flaxseed oil or 10% corn oil in modified AIN-93G diet from postnatal day 28 to 91. Feed intake was recorded every 2 or 3 days and body weights were recorded weekly. On postnatal day 91, the mice were asphyxiated with CO_2 and blood samples were collected by cardiac puncture. Serum interleukin-1β, interleukin-6, and tumor necrosis factor-α (TNF-α) levels were determined using enzyme-linked immunoassays. The serum fatty acid profile (ALA, linoleic acid, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid) was determined using gas chromatograph/mass spectrometry. The right femurs of the mice were removed, cleaned of soft tissue, and stored at -70°C until analyzed. Biomechanical bone strength testing was conducted on the femurs using a Materials Testing System followed by the generation of load-deformation curves using specialized computer software. For determining peak load, compression testing was conducted on individual lumbar vertebrae (LV3). Bone mineral content and density of left femurs and lumbar vertebrae (LV1-LV4) were determined using PIXImus dual-energy X-ray absorptiometry.

Feed intake, body weights, and serum cytokines did not differ between the corn oil and flaxseed oil treatment groups. Mice from the flaxseed group had statistically significantly higher serum ALA, eicosapentaenoic acid, and docosahexaenoic acid levels but significantly lower serum linoleic acid and arachidonic acid levels than mice fed corn oil. There was no difference between the sexes in serum ALA, eicosapentaenoic acid, and linoleic acid levels; however, serum arachidonic acid and docosahexaenoic acid levels were significantly higher in males than females of the flaxseed group. The peak load of LV3 and the bone mineral content and density did not differ between the 2 diet groups; however, bone mineral content and density were markedly (statistical significance not specified) higher in male mice. LV3 peak load was similar between the sexes. Femur dimensions and biomechanical bone strength properties were similar between the diets. Males tended to have greater femur dimensions and biomechanical bone strength compared to females. The results of this study show that feeding 10% flaxseed oil in the diet of growing mice does not affect bone development. The authors concluded that, "These findings suggest that neither bone mineral nor matrix of femur and lumbar vertebrae was improved or compromised in growing male and female mice fed a 10% flaxseed oil diet compared to a 10% corn oil diet. This level of ALA that is attainable from a 10% flaxseed oil

diet is considered safe with respect to biomechanical bone strength and bone mass int his mouse model."

4.2.3.3. Summary and Conclusions from Developmental Reproduction Toxicity Studies

A total of 14 published studies has been directed toward the assessment of the developmental and reproductive toxicity of flaxseed, flaxseed meal, or a constituent, either α-linolenic acid (ALA) or SDG. All but one of these studies were conducted in rats, most in Sprague-Dawley rats; one study of flaxseed oil was conducted with Sv/Ev mice. The flaxseed levels used in these studies ranged from 5% dietary concentration (approximately 3750 mg/kg bw/day) to 40% dietary concentration (approximately equivalent to 30,000 mg/kg bw/day).

Studies in which flaxseed was administered at 5% dietary concentration found either no effect (Tou and Thompson 1997; Orcheson et al. 1998) or effects at 5% dietary concentration with opposite effects at 10% concentration (Tou et al. 1998a; Tou et al. 1998b; Tou and Thompson 1999; Tou et al. 1999), discussed further below. A number of studies also found no effect with flaxseed at 10% of the diet (Orcheson et al. 1998; Ward et al. 2001a; Hemmings and Barker 2004). An absence of effects has been observed at even higher doses; Wiesenfeld et al. (2003) found no effects other than non-adverse changes in fatty acid absorption in SD rats given flaxseed meal (defatted flaxseed) at 13% or 26% of the diet or flaxseed at 20% or 40% of the diet. Other studies assessing specific adverse effects have also found an absence of adverse effects from flaxseed even at high concentrations; Sprando et al. (2000b) found no effect of flaxseed on spermatogenesis or testis structure at either 20% or 40% of the diet. Ward et al. (2001b) and Cohen and Ward (2005) showed that bone development is unaffected by ingestion of 10% flaxseed, 10% flaxseed oil, or the lignans present in a 10% flaxseed diet.

However, other studies of higher dietary concentrations of flaxseed have found estrogenic or antiestrogenic effects. These effects often appear to be contradictory, with one effect appearing at one dose and the opposite effect appearing at a different dose, particularly in the studies by Tou et al. Examples of such contradictory effects include:

- decreased prostate weights with flaxseed at 5% dietary concentration, increased prostate weights at 10% (Tou et al. 1998a)
- female rats had delayed puberty with flaxseed at 5% and premature puberty at 10% (Tou et al. 1998a)
- relative ovary weights were decreased at 5% flaxseed and increased at 10% (Tou et al. 1998b)
- female rats had delayed puberty with flaxseed at 5% and premature puberty at 10% (Tou et al. 1998b)
- female rats had delayed puberty at 5% and premature puberty at 10% (Tou et al. 1999)
- female rats had delayed puberty with flaxseed at 5% and premature puberty at 10% (Tou and Thompson 1999)

Tou et al. (1999) suggested that the dose, timing, and duration of flaxseed exposure all affect its hormonal effects and concluded that lower concentrations tend to produce antiestrogenic effects while higher concentrations produce estrogenic effects. Although no adverse effects were seen on growth, clinical chemistry, hematology, or histopathology at any dose, flaxseed appears to frequently produce weak estrogenic effects at high levels of intake even though these effects seem to be inconsistent from study to study.

4.2.4. Special Study

Because flaxseed contains high concentrations of ALA, which may inhibit lymphocyte function in both rats and humans, a study was conducted using Sprague-Dawley rats to examine the potential effect of flaxseed or flaxseed meal on spleen fatty-acid profiles, changes in splenic mononuclear cell subpopulations, and mitogen-induced proliferation and interleukin-2 production by mononuclear cells (Babu et al. 2003). Pregnant rats were individually housed in stainless steel hanging cages and fed throughout gestation with diets containing 0% flaxseed or flaxseed meal (Purina Rodent Chow 5002M), 20% flaxseed, 40% flaxseed, 13% flaxseed meal, or 26% flaxseed meal. Some of the rats had a Caesarean section on gestation day 20 and others were allowed to litter and rear their young. Post-weaning pups were maintained on the same diet regime as their dams. Mononuclear cells were isolated from the spleen of pregnant rats and 90-day-old F_1 generation rats. Splenic lymphocyte populations were analyzed using a flow cytometer. Mononuclear cell proliferation was measured *in vitro* after 72-hour incubation with varying concentrations of concanavalin A, phytohemagglutinin, or lipopolysaccharide. Interleukin-2 concentrations were determined in concanavalin A and phytohemagglutinin-stimulated supernatants taken from splenic mononuclear cell incubations. Lastly, the fatty-acid profiles of spleen mononuclear cells were determined using GLC.

Ingestion of flaxseed or flaxseed meal did not affect splenic CD4, CD8, MAC1 or IA positive cell populations. There were a few differences in the populations between pregnant and F_1 generation animals; pregnant rats had a significantly higher CD4 population and significantly lower CD8, MAC1, and IA-positive populations. The only statistically significant mitogenic response of spleen cells was in animals fed 40% flaxseed. Pregnant animals showed a decreased response to concanavalin A (~60% lower) and F_1 animals showed a decreased response to phytohemagglutinin (~51% lower) when compared with controls. Interleukin-2 production was not affected by dietary administration of flaxseed or flaxseed meal to pregnant or F_1 rats. Splenic fatty-acid profiles were changed by ingestion of flaxseed or flaxseed meal. The most notable changes were as follows (compared to controls): ALA was significantly increased in pregnant and F_1 rats fed flaxseed or flaxseed meal; linoleic acid was significantly lower in pregnant and F_1 rats fed 40% flaxseed; and arachidonic acid was significantly lower in pregnant and F_1 rats fed either flaxseed or flaxseed meal. This study does not call into question conclusions reached on the basis of the studies previously discussed. The authors noted that although splenic concentrations of fatty acids, including ALA, linoleic acid, and arachidonic, were affected by the high doses of flaxseed and flaxseed meal tested in this study, no significant changes were observed in the cell subsets or IL-2 production by splenic cells from the different treatment groups.

4.2.5. Genotoxicity/Mutagenicity Studies

The mammalian lignans enterolactone and enterodiol and plant lignans matairesinol and secoisolariciresinol were tested in the microtubule assembly assay and for growth inhibition, cytotoxicity, mitotic arrest, tubulin staining, CREST[1] staining, micronuclei, and HPRT mutation in cultured male Chinese hamster V79 cells (Kulling et al. 1998). At concentrations of 200 μM, none of the lignans inhibited cell-free microtubule assembly. Similarly, at concentrations of 100 μM, the lignans had no effect on cytoplasmic microtubule complex in interphase cells and the mitotic spindle in metaphase cells. Enterolactone, enterodiol, and secoisolariciresinol produced only slight cell growth inhibition at a concentration of 100 μM; while matairesinol inhibited cell proliferation to the same extent as the positive control (diethylstilbestrol). None of the lignans affected cell morphology or produced significant cytotoxicity at this concentration. When incubated for 6 hours with V79 cells, no significant increase in the number of metaphase cells were noted for up to 100 μM enterolactone, enterodiol, or secoisolariciresinol when compared with control values. Matairesinol tended to decrease the number of mitotic cells. None of the lignans (tested at 50 and 100 μM) induced micronuclei (whole or fragmented) after incubation for 6 hours with V79 cells followed by incubation in fresh medium for 3-24 hours. CREST staining was used to distinguish between micronuclei containing whole chromosomes and those containing chromosomal fragments. In the HPRT forward mutation assay, none of the lignans (tested at 50 and 100 μM) induced a significant increase in 6-TG-resistent cells over control values. The authors concluded that enterolactone, enterodiol, matairesinol, and secoisolariciresinol showed no potential for aneuplidogenic, clastogenic, or mutagenic activity.

The National Toxicology Program (NTP) tested linseed oil (CAS No. 8001-26-1) at concentrations of 10, 33, 100, 333, 1000, and 3333 μg/plate in a standard Salmonella preincubation assay using *Salmonella typhimurium* strains TA100, TA1535, TA97, and TA98 with and without metabolic activation (NTP 1991). Metabolic activation was derived from induced male Sprague Dawley rat or Syrian hamster liver S9 and cultures containing 10 or 30% of either metabolic activation source were tested. Precipitate was noted in most cultures of 333 μg/plate or higher. Negative results were reported in all strains with or without metabolic activation, indicating no evidence of genetic toxicity.

Rojas-Molina et al. (2005) tested the genotoxicity of 7 vegetable oils in the *Drosophila* wing-spot test, including flaxseed, soy, extra-virgin olive, low-grade olive, sunflower, sesame, and wheat-germ oils. Larvae of the standard and highly bioactive crosses of *Drosophila melanogaster* were fed medium containing 6% or 12% of each of the test oils. Urethane was used as a positive control. At the lower (6%) concentration, none of the oils tested positive in both the standard and bioactive cross. Flaxseed oil produced a positive response in the standard cross, but not in the highly bioactive cross. At the higher (12%) concentration, flaxseed, soy, sesame, and wheat-germ oils produced positive responses in the standard crosss, and all except soy oil were also positive in the bioactive cross. The experimenters concluded that the positive results were due to high concentrations of polyunsaturated fatty acids, which are known to be associated with the production of oxidative DNA damage.

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[1] Calcinosis, Raynaud's phenomenon, esophagal motility abnormalities, sclerodactyly and telangiectasia

Finally, Roberts et al. (2006) reported that 7-hydroxymatairesinol was not genotoxic in the Ames assay, the *in vitro* chromosome aberration assay, or the *in vivo* rat bone-marrow micronucleus assay.

In summary, flaxseed oil showed no indication of genotoxicity in reverse bacterial mutation (Ames) assays or in the *Drosophila* wing-spot test. Micronucleus testing also showed the primary lignans found in flaxseed, secoisolariciresinol and matairesinol, as well as the mammalian lignans enterolactone and enterodiol, to non-aneuplidogenic, non-clastogenic, and non-mutagenic.

4.2.6. Other Animal Studies

In a study available only in abstract form, Siemelink et al. (2001) rats (number and strain not reported) were placed on diets supplemented with flaxseed oil, coconut oil, safflower oil, soybean oil, or menhaden oil beginning 2 weeks prior to mating; the diets were continued through pregnancy. After weaning, the pups were given the same diets as their dams until the age of 12 weeks. There were no differences in the number of fetuses, corpora lutea, resoptions, or uterus weights, nor were any effects seen on the weights of the fetuses or placentas. Weight gain of the dams during pregnancy was lower among the rats receiving menhaden oil but did not differ among the remaining groups. Similarly, pups born to dams receiving menhaden oil had lower birth weights than did those in the other groups, which did not differ among themselves, as well as lower growth. It was concluded that flaxseed oil, as well as coconut oil, safflower oil, and soybean oil, had no effect on pregnancy or early growth.

Another study available only as an abstract (published in Serbian) was an investigation into the effect of dietary factors on histologic changes and metabolism in kidneys in an experimental model of polycystic kidney disease (Bankovic-Calic et al. 2002). Male offspring of Han:SPRD-cy heterozygotic rats received *ad libitum* diets containing 8 or 20% casein, 20% soy protein, or 10% flaxseed for 8 weeks. After sacrifice, tissue was harvested for histological assessment and analysis of metabolic changes in lipids, citric-acid metabolites, and osmolytes. Measures were taken of cystic changes, renal tubular proliferation and apoptosis, numbers of interstitial cells and macrophage infiltration, and interstitial fibrosis. The animals fed flaxseed (at approximately 7500 mg/kg bw/day) had decreased cystic size and less interstitial inflammation and fibrosis; there was no effect on epithelial cell apoptosis or proliferation. Significant renal enrichment of n-3 polyunsaturated fatty acids was observed, along with increased betaine and succinate. Urinary citrate excretion was increased without any change in ammonia excretion. These changes were beneficial in ameliorating Hans:SPRD-cy rat polycystic kidney disease with no apparent adverse effects.

Velasquez et al. (2003) used a spontaneously hypertensive/NIH-corpulent (SHR/N-cp) rat model to study the effects of casein, soy, or flaxseed on renal function and nephropathy. Obese 8-10-week-old male SHR/N-cp rats were randomly assigned to receive diets (10 rats/group) containing 20% casein, soy protein concentrate, or flaxseed meal for 6 months. The flaxseed meal contained 19.0 mg SDG/g. Feed intake and body weight were measured weekly. At the end of the feeding period the rats were placed in metabolic cages and fasting urine collections

were made, after which the rats were sacrificed and blood taken for biochemical analysis. After saline perfusion, the kidneys were dissected, weighed, and subjected to histological examination. Serum was analyzed for glucose, protein, urea, and creatinine; urine was analyzed for the last 3 parameters.

All 3 diets were well tolerated and neither feed intake nor final body weights differed among the 3 groups. Plasma glucose and creatinine levels did not differ, but plasma insulin was lower in the flaxseed-meal group. There were no differences in urinary creatinine or urea clearance, but protein excretion was lower in the flaxseed-meal group. The rats receiving flaxseed meal had the lowest incidence of abnormal glomeruli with mesangial expansion and the lowest tubulointerstitial scores. The authors concluded that this study demonstrated that consumption of flaxseed meal exerts a renal protective effect with no indication of any adverse effects.

In a study of the effect of flaxseed meal on hypertriglyceridemia and steatosis of the liver in an animal model of obesity, Bhathena et al. (2003) subjected lean and obese male SHR/N-cp rats to 2 weeks of acclimatization. By this age the lean rats weighed about 96 g while the obese rats weighed about 125 g. Rats were then assigned to 3 groups (10 lean and 10 obese rats/group) to receive rat chow supplemented with 20% of energy from casein, soy protein concentrate, or flaxseed meal (containing 17.0 mg SDG/kg) for 6 months. Feed intake and body weight were measured biweekly. After sacrifice, blood was collected and plasma was separated for analysis of total cholesterol, HDL cholesterol, triacylglycerol, and total protein, and the liver was removed, weighed, and processed for histopathological examination.

The obese rats ate more than did the lean rats. There were no differences between groups in the final body weights or liver weights of lean rats. As compared to rats fed casein, those fed flaxseed meal or soy protein concentrate had lower plasma total cholesterol and those fed flaxseed meal had lower levels of triacylglycerols. The obese rats had higher body and liver weights than did the lean rats, along with elevated plasma concentrations of total cholesterol, HDL cholesterol, LDL cholesterol, and triacylglycerols, but similar levels of total protein. However, the liver weights of obese rats fed flaxseed meal were significantly lower than rats in the other feeding groups, as were plasma total protein levels, total cholesterol, HDL and LDL cholesterol, and triacylglycerols. Livers of obese rats were more likely to exhibit histopathology than those of lean rats, but there were no differences across feeding groups. No adverse effects were noted, and the authors concluded that flaxseed meal supplementation may provide a new therapeutic strategy to reduce hypertriglyeridemia and fatty liver.

Mueller et al. (2004) studied the effect of n-3 fatty acids from different sources on canine atopic dermatitis in a randomized, blinded, placebo-controlled study with 29 dogs that received feed supplemented with 200 mg/kg bw/day flaxseed oil, 50 mg EPA and 35 mg DHA/kg bw/day, or mineral oil for 10 weeks. Although the dogs' clinical scores improved with consumption of flaxseed oil or EPA+DHA as compared to mineral oil, there was no correlation between total fatty acid intake or n-6:n-3 ratio and clinical scores. No adverse effects were reported.

Table 32. Safety Studies in Animals

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Babu et al. 2003	Investigate the effects of ingestion of dietary flaxseed and of defatted flaxseed meal on immune function, including fatty acid composition, cell subsets, proliferation, and IL-2 production by splenic lymphocytes.	Animals were mated and randomly assigned to 1 of 5 feeding groups. 10 pregnant rats per group were euthanized at gestation day 20; any pups >10 were culled at postnatal day 4. Pups suckled to postnatal day 21-23, then fed respective diet.	Pregnant Sprague-Dawley rats and their pups	Control or 1 of 4 test diets: 13% or 26% flax meal; 20% or 40% flaxseed; equivalent to 9750 or 19,500 mg/kg bw/ day flaxseed meal and 15,000 or 30,000 mg/kg bw/ day flaxseed	From impregnation to gestation (c. 21-23 days) and until postnatal day 90	In pregnant rats, the FS-fed rats, v. the FM-fed rats, had higher spleen-cell SFA, MUFA (oleic), EPA, and ALA and lower PUFA (linoleic) and AA; in F_1 rats, the FM-fed rats, v. controls, had higher spleen-cell SFA; the FS-fed rats had higher MUFA, EPA, and ALA and lower AA Among pregnant rats, spleen cell proliferation in response to Con A was significantly lower in the high-FS group, but there were no significant differences in PHA- or LPS-mediated proliferation. In the F_1 rats, PHA-mediated spleen cell proliferation was lower in the high-FS group, but there were no significant differences in proliferation mediated by Con A or LPS. No effects on IL-2 production were seen in either pregnant or F_1 rats, and dietary treatment did not affect splenic CD4, CD8, MAC1, or IA positive cell populations. CONCLUSION: No adverse effects were seen. The effect on lymphocyte proliferation is due to the fatty-acid component and is an effect common to LC-PUFAs.
Bankovic-Calic et al. 2002	Effect of flaxseed, low protein, and soy on renal injury	Flaxseed v. control; studied effect on histology and metabolism	Male Han:SPR D-cy rats	10% flax-seed in diet; equivalent to 7500 mg/kg bw/day	8 weeks	Histology: moderate decrease in cystic size; less interstitial inflammation and fibrosis; no difference in epithelial cell apoptosis and proliferation. Metabolism: increase in renal content of n-3 fatty acids, betaine, and succinate; increased urinary citrate excretion; no change in urinary ammonia excretion. CONCLUSION: All differences were seen as beneficial rather than adverse.

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Table 32. Safety Studies in Animals

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Bhathena et al. 2003	Effect of flaxseed and soy protein on hypertriglyceri-demia and liver steatosis in obesity	Corpulent v. lean rats fed diets with 1 of 3 supplements: casein, soy protein concentrate, or flaxseed meal	SHR/-cp rats	Flaxseed meal provided 20% of energy; contained 17.0 mg/kg secoisolarici-resinol	6 months beginning at 7-8 weeks of age	No difference was seen in feed intake or weight gain in either lean or obese rats; no difference was seen in absolute or relative liver weights in lean rats, but reductions in both were noted in obese rats as well as lower plasma protein, total cholesterol, HDL, LDL, and triglyceride levels. There were no differences in liver histology, although lower grades of liver fat were noted in both lean and obese rats. CONCLUSION: All differences were seen as beneficial rather than adverse; no adverse effects were seen or reported.
Cohen and Ward 2005	Investigate the effects of flaxseed intake on bone development in male and female mice	Male (n=14-22 mice/ group) and female (n= 10-19 mice/ group) mice were fed flaxseed oil or corn oil in their diet	Weanling 129 Sv/Ev mice	10% flax-seed oil or 10% corn oil in modified AIN-93G diet	63 days from postnatal day 28 to 91	Feed intake, body weights, and serum cytokines did not differ between the corn oil and flaxseed oil treatment groups. Mice from the flaxseed group had higher serum ALA, EPA, and DHA levels but lower linoleic and arachidonic acid levels than mice fed corn oil. There was no difference between the sexes in ALA, EPA, or linoleic acid levels but arachidonic acid and DHA levels were higher in males than females of the flaxseed group. Bone mineral content and density did not differ between the two diet groups. Femur dimensions and biomechanical bone strength properties were similar between the diets. CONCLUSION: The results of this study show that feeding 10% flaxseed oil in the diet of growing mice does not affect bone development.

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Table 32. Safety Studies in Animals

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Collins et al. 2003	Investigate the effects of ingestion of dietary flaxseed and of defatted flaxseed meal. This article: effects on reproduction and development.	Animals were mated and randomly assigned to 1 of 5 feeding groups. 10 pregnant rats per group were euthanized at gestation day 20; any pups >10 were culled at postnatal day 4. Pups suckled to postnatal day 21-23, then fed respective diet.	Pregnant Sprague-Dawley rats and their pups	Control or 1 of 4 test diets: 13% or 26% flax meal;, 20% or 40% flaxseed; equivalent to 9750 or 19,500 mg/kg bw/ day flaxseed meal and 15,000 or 30,000 mg/kg bw/ day flaxseed	From impregnation to gestation (c. 21-23 days) and until postnatal day 90	All F_0 and F_1 animals appeared healthy and no compound-related external effects were noted. FS did not affect length of gestation, but FM decreased it dose-responsively. There were no differences in feed consumption. In the teratology phase, there were no effects on numbers of corpora lutea, implants per female, viable male and female fetuses, early or late deaths, fetal weights, anogenital distances, external variations, fetal bone or soft-tissue development. In the reproduction phase, no significant effects were seen on survival to weaning, animals born, animals live born, or animals alive at PND 4; no differences in weight gain to PND 14, but dose-related decreases in weight gain to PND 21 in both FS and FM groups. No effects on body weights and absolute organ weights of F_1 males or organ weights of F_1 females. Relative to body weight or brain weight, some decreases were seen in weights of thymus, liver, and spleen. During PND 21-90, neither F_1 males or females showed any dose-related clinical effects. No effect on weight gain in males, but FM-fed females gained more weight than controls. Absolute organ weights were not affected, but some differences were seen in relative organ weights. Puberty of females was not affected, but was delayed in FM-fed males. FM-fed females were more likely to have irregular estrous cycles. No histopathology was evident. CONCLUSION: FS and FM at these doses did not adversely affect the estrogen balance. No differences were apparent in developmental toxicity parameters at the time of C section. EPA metabolite levels were not sufficiently increased to affect luteal function. Changes in fatty acids were insufficient to cause hemorrhages. Acyclic females were expected, but this was not observed. There were no apparent effects on pregnancy and no differences in the weights of male and female offspring at birth. Overall, no effect on fertility or fetal development, was observed, but hormone-related effects on the offspring were seen during postnatal development and sexual maturation.

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Table 32. Safety Studies in Animals

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Farmer et al. 2007	Study the effect of consumption of dietary flaxseed on fatty acid profile, prepubertal development of mammary glands, and bone resorption in piglets	Piglets were fed basal diet , diet containing 10% flaxseed, diet containing 6.5% flaxseed meal (defatted flaxseed, or diet containing 3.5% flaxseed oil; mated at 151 days of age	Yorkshire x Landrace gilts (female piglets) aged 82 days	10% flax-seed (equivalent to 2700 mg/ kg bw/day or about 1750 mg/kg bw/day flax-seed meal or about 950 mg/kg bw/day flax-seed oil	125 days (to age 212 days)	Dietary supplementation with flaxseed products did not affect body weight, back fat thickness, number of corpora lutea, 5-bromo-2'-deoxyuridine labeling index, estrogen receptor localization, or plasma concentrations of estradiol, prolactin, or cross-linked N-telopeptides of type I collagen. Ingestion of 10% flaxseed or 3.5% flaxseed oil, but not 6.5% flaxseed meal, increased the concentration of PUFA and decreased the concentrations of SFA and MUFA in plasma and extraparenchymal tissue. There was an increase in the dry matter content of mammary tissue in animals fed flaxseed. CONCLUSION: Flaxseed and flaxseed oil, but not defatted flaxseed meal affected fatty acid profiles; none produced any effect on mammary development or bone resorption. The NOAEL for flaxseed was 2700 mg/kg bw/day. The NOAELs for flaxseed meal and oil were 1750 and 950 mg/kg bw/day, respectively.
Flynn et al. 2003	Investigate the effects of ingestion of dietary flaxseed and of defatted flaxseed meal on cultured embryos.	Animals were mated and randomly assigned to 1 of 5 feeding groups. 10 pregnant rats per group were euthanized at gestation day 20 and blood was collected; serum was extracted. Rat embryos were cultured in a medium infused with serum.	Pregnant Sprague-Dawley rat serum and S-D rat embryos	Control or 1 of 4 test diets: 13% or 26% flax meal; 20% or 40% flaxseed; equivalent to 9750 or 19,500 mg/kg bw/ day flaxseed meal and 15,000 or 30,000 mg/kg bw/ day flaxseed	NA	No consistent dose-related effects were seen in any of 4 classes of abnormalities: situs inversus-related, neural tube defects, flexion defects, or other defects. CONCLUSION: Serum from rats exposed to high levels of FS or FM is not embryotoxic and not teratogenic. "Flaxseed and flax meal diets, at levels up to 40% and 26% respectively, do not alter the toxicological or nutritional properties of rat serum in any way that suggests that such diets would pose a developmental hazard through disruption of organogenesis.

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Table 32. Safety Studies in Animals

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Hemmings & Barker 2004	Evaluate the safety of flaxseed consumption.	On day 18 of gestation, pregnant rats were put on a diet of rat chow with 10% flaxseed or control. Pups were weaned on PND 31 and put on same diet as dams. Pups were sacrificed on days 10, 28, 48, and 86.	M&F Fischer 344 rats	10% flax-seed in the diet, equivalent to 7500 mg/kg bw/day	Prenatally and until PND 86 with weaning on PND 31 (55 days feeding + 31 days nursing flaxseed-fed dam)	There was no effect on body weight or body weight gain and no effect on righting reflex, pinna detachment, eye opening, or acoustic startle. No effect was noted in relative organ weights and no visible differences in organs and no differences in plasma ALT or γGT although a slight increase was seen in activity (regarded as a beneficial effect). There was no effect on blood glucose level. The increase in γGT activity may indicate an estrogenic effect. CONCLUSION: Dietary 10% flaxseed (approximately 7500 mg/kg bw/day) is without long-term effect on growth, development, or behavior; is non-toxic; and may be hepatoprotective.
Hemmings et al. 2004	Further investigate the apparent estrogenic effect noted in Hemmings and Barker 2004.	At age 30 d, male rats were divided into 3 groups (7-9 rats group): 10% high-ALA Norlin flax-seed, 10% low-ALA Solin flax- seed, control. Sacrificed on PND 80 and 130.	Male Fischer 344 rats	10% flax-seed in the diet, equivalent to 7500 mg/kg bw/day	From PND 30 to PND 130 (100 days)	There were no differences among the groups in growth, and no physical or behavioral differences. No gross changes were seen in organs and no differences in relative organ weights. There was no difference in ALT or in glucose. γGT activity in the liver, but not the kidney, was significantly higher in the flaxseed-fed rats, but no difference was observed between high-ALA and low-ALA flaxseeds. CONCLUSION: Confirmed previous finding that flaxseed feeding at 10% dietary concentration (approximately 7500 mg/kg bw/day) is safe and produces no adverse effects. The increase of γGT activity appears to be due to the lignan rather than ALA, most likely SDG.

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Table 32. Safety Studies in Animals

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Lina et al. 2005	Evaluate the oral subchronic toxicity of 7-hydroxy-matairesinol (7-HMR, closely related to the matairesinol found in flaxseed) in the rat.	Rats were fed levels of 0, 0.25, 1, and 4% of a potassium acetate complex of 7-HMR, resulting in an average daily intake of 0, 160, 640, and 2600 mg HMR/kg bw/ day for 90 days	Wistar rats	Potassium acetate complex of 7-HMR at 0, 0.25, 1, or 4% dietary concentra-tion, equivalent to 0, 160, 640, or 2600 mg/ kg bw/ day	90 days	Dose-related increases were seen in the plasma concentrations of 7-HMR and its metabolites (chiefly enterolactone), but there were no effects on clinical signs, ophthalmoscopy, neurobehavioral observations, or motor activity. Some reduction in feed intake and body weight gain was observed in the mid- and high-dose groups, which was ascribed to decreased palatability of the test feed. There were isolated differences in white blood cell counts, plasma triacylglycerols, and phospholipids. The only effect seen that was regarded as adverse was decreased ovary weights seen in mid-and high-dose females. CONCLUSION: Based on reduced ovary weights, the NOAEL of 7-HMR was set at 0.25% dietary concentration, corresponding to 160 mg/kg bw/day intake.
Mueller et al. 2004	Evaluate the effect of n-3 fatty acids (ALA, EPA, DHA) on canine atopic dermatitis.	29 dogs received 1 of 3 treatments: flaxseed oil, EPA+DHA, or mineral oil as a placebo.	Dogs	200 mg/kg/d	10 weeks	No indication of any adverse effects; dogs' clinical scores improved in both test groups relative to control.
Orcheson et al.1998	Assess the effect of dietary flaxseed administration on estrous cycling in rats	Rats were assigned to receive1 of 8 treatments: high-fat basal diet; basal diet + 0.75, 1.5, or 3.0 mg SDG; basal diet + 2.5, 5, or 10% flax-seed; or basal diet + tamoxifen	Sprague-Dawley rats aged 58 days	0.75, 1.5, or 3.0 mg SDG; 0, 2.5, 5, or 10% flax-seed, equivalent to 0, 1875, 3750, or 7500 mg/kg bw/day	4 weeks	66% of the high flaxseed- and high SDG-dose rats were either acyclic or irregular vs.16% in the basal diet group. Rats on flaxseed and SDG tended to have longer cycles, but this was significant only for the 5% flaxseed group and was not dose-dependent. Urinary excretion of lignans was higher in flaxseed than SDG groups. Rats given flaxseed or SDG did not differ from controls in body weight, feed intake, organ weights (ovaries, liver, heart, kidneys, spleen, cecum, and colon), or gross pathology. CONCLUSION: Administration of flaxseed or SDG was well tolerated and produced no inherent toxicity in rats at the doses tested, equivalent to 1900, 3750, and 7500 mg/kg bw/day of flaxseed.

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Table 32. Safety Studies in Animals

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Prasad 2005	Investigate the safety of flaxseed with regard t the hemopoietic system.	Administer lignan complex isolated from flaxseed to normo- and hypertensive rabbits v. un-supplemented rabbit chow.	Female New Zealand white rabbits weighing 1.2-1.5 kg	40 mg/kg bw/day lignan complex (equivalent to the amount found in about 3000 mg/kg bw/d of flaxseed)	60 days	No adverse effects on counts of red blood cells, white blood cells, granulocytes, lymphocytes, monocytes, or platelets, nor on mean corpuscular volume, red cell distribution width, hematocrit, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, or platelet volume. CONCLUSION: Chronic use of lignan complex had no adverse effects on the hemopoietic system.
Ratnayake et al. 1992	Investigate the nutritional effects of diets supplemented with high levels of flaxseed	Animals randomly assigned to 4 dietary treatment groups	Weanling male Sprague-Dawley rats	0%, 10%, 20%, or 40% flaxseed; equivalent to 0, 7500, 15,000 or 30,000 mg/kg bw/ day	90 days	Flaxseed was oxidatively and thermally stable. No effects were observed in feed consumption,: body weight, or organ weights, nor on glucose, but triacylglycerols, total cholesterol and LDL were lowered dose-dependently; HDL and vitamin E were lowered only in the 40% flaxseed group. With regard to fatty acids in tissues, the changes reflected the composition of the dietary fats and conversion of ALA to other n-3 PUFAs (mainly EPA). There was no effect on Zn despite high phytate intake.Ssoftening of stools was see at all dose levels. CONCLUSION: no adverse effects were seen on growth or nutritional status. No adverse effects were observed in the rats fed flaxseed, even at high intakes.

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Table 32. Safety Studies in Animals

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Siemelink et al. 2001	Study the effects of different diets during pregnancy and weaning on parameters related to chronic diseases during adulthood.	Beginning 2 weeks before pregnancy, fed rats diets supplemented with coconut oil, safflower oil, soybean oil, menhaden oil, or flaxseed oil. Continue the feeding to the pups.	Rats	20% dietary concentration of flaxseed oil, equivalent to 15,000 mg/kg bw/day	Begin 2 weeks before impregnation and continue through pregnancy and to pups after weaning to age 12 weeks	There were no differences in the number of fetuses, corpora lutea, resorptions, or uterus weights. No effect of the oils on weights of fetuses or placentas, no differences in feed consumption or weight gain during pregnancy (except menhaden group was lower). No effect on birth weight or growth of the pups (except menhaden group was lower). CONCLUSION: Flaxseed oil, as well as coconut oil, safflower oil, and soybean oil, had no effect on pregnancy or early growth.
Sprando et al. 2000	Investigate the effects of ingestion of dietary flaxseed and of defatted flaxseed meal on spermatogenesis and endocrine function in rats	Animals were mated and randomly assigned to 1 of 5 feeding groups. 10 pregnant rats per group were euthanized at gestation day 20; any pups >10 were culled at postnatal day 4. Pups suckled to postnatal day 21-23, then fed respective diet.	Pregnant Sprague-Dawley rats and their pups	Control or 1 of 4 test diets: 13% or 26% flax meal (FM); 20% or 40% flaxseed; equivalent to 9750 or 19,500 mg/kg bw/ day flaxseed meal and 15,000 or 30,000 mg/kg bw/ day flaxseed	From impregnation to gestation (c. 21-23 days) and until postnatal day 90	No effect was seen on testis or epididymal weights or on absolute or relative counts of homogenization-resistant spermatids. Mid-dose FS diet and both FM diet rats had lower prostate weight, but no significant effect was seen on on seminal vesicle weight. There were dose-related increase in LH in the FS-treated rats and in the high-dose FM group, which also had elevated testosterone, and both cauda epididymal weights and cauda sperm counts increased dose-dependently in the FS-diet groups. There was no effect on sperm morphology. Treated rats had a full complement of germ cells and normal-appearing cell associations in each tubule and inter-tubularly, and non-remarkable seminiferous epithelia; no histopathology was seen CONCLUSION: Exposure to flaxseed or flax meal at these levels resulted in a decrease in prostate weight and an increase in serum LH and cauda epididymal sperm numbers, likely due to the lignans; these may be regarded as adverse reproductive effects.

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Table 32. Safety Studies in Animals

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Sprando et al. 2000b	Assess the potential of flaxseed to affect spermatogenesis in rats	Animals were mated and randomly assigned to 1 of 5 feeding groups. 10 pregnant rats per group were euthanized at gestation day 20; any pups >10 were culled at postnatal day 4. Pups suckled to postnatal day 21-23, then fed respective diet for 70 days	Pregnant Sprague-Dawley rats and their pups	Control or 1 of 4 test diets: 13% or 26% flax meal (FM); 20% or 40% flaxseed; equivalent to 9750 or 19,500 mg/kg bw/ day flaxseed meal and 15,000 or 30,000 mg/kg bw/ day flaxseed	From impregnation to gestation (c. 21-23 days) and until postnatal day 90	No differences were found in body weights or testis weights in any of the groups. Volumes of the seminiferous tubules in rats receiving flaxseed were lower than in the controls, but differences were not observed in Sertoli cell nucleolar numbers, nor in the length or surface areas of the seminiferous tubules. Flaxseed and flaxmeal did not adversely affect spermatogenic activity in the treated animals and the statistically significant differences in volume were not biologically significant. The findings of this study confirm those of Sprando et al. (2001a). CONCLUSION: Flaxseed and flaxmeal do not affect spermatogenesis or testis structure in the rat at the doses tested in this study, approximately equivalent to 9750 and 19,500 mg/kg bw/day of flaxseed meal and 15,000 and 30,000 mg/kg bw/day of flaxseed.
Tou & Thompson 1997	Determine if flaxseed affects reproduction and development.	Fed basal diet or basal diet supplemented with flaxseed at 5% or 10% to rats during pregnancy, pregnancy and lactation, weaning to PND 50, weaning to PND 132, or pregnancy through pup adulthood.	Rats	5% or 10% flaxseed dietary con-centration, equivalent to 3750 or 7500 mg/kg bw/day	During pregnancy, pregnancy and lactation, weaning to PND 50, weaning to PND 132, or dam pregnancy through pup adulthood	Pregnancy: no effect on gestation length, parturition, litter size, pup birth weight (except reduction at 10%), and survival. During suckling, 10% flaxseed decreased pup growth, shortened the anogenital distance, and increased pups' ovarian weight. Flaxseed after weaning had no effect on onset of puberty in females, but increased ovarian weight premature vaginal opening in females and increased prostate, seminal vesicles, accessory sex gland, and testes weight in males. CONCLUSION: At high dietary levels, flaxseed can cause hormonally related effects in the offspring.

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Table 32. Safety Studies in Animals

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Tou et al. 1998a	Determine the effect of timing of flaxseed feeding on reproductive effects.	Fed basal diet or basal diet supplemented with either 5% or 10% flaxseed to pregnant rats; on weaning, fed flaxseed to offspring whose dams had received basal diet, and basal diet to offspring whose dams had received flaxseed.	Rats	5% or 10% flaxseed dietary con-centration, equivalent to 3750 or 7500 mg/kg bw/day	During pregnancy or from weaning until ?	No effect was seen from flaxseed fed after weaning, but.dose-dependent effects from flaxseed fed during pregnancy and lactation were observed. Perinatal 5% flaxseed: delayed puberty and produced diestrus in female offspring and an elevated estradiol:testosterone ratio and decreased prostate weight in male offspring. Perinatal 10% flaxseed: increased serum estradiol, premature irregular cycles, and persistent estrus in female offspring, and elevated testosterone and increased prostate weight in male offspring. Some of these effects (e.g., estradiol and prostate weight) are inconsistent and may not represent reproductive effects. CONCLUSION: No effects were seen due to flaxseed exposure at either 5 or 10% of the diet fed after weaning, but perinatal exposure resulted in several effects that were dose-dependently paradoxical with opposite effects at different doses.
Tou et al. 1998b	Determine effects of flaxseed intake and intake of secoiso-lariciresinol diglyco-side (SDG) during pregnancy and lactation on reproductive development.	Fed basal diet or basal diet supplemented with 5% or 10% flaxseed or SDG at the equivalent of 5% flaxseed to rats during pregnancy and lactation.	Sprague-Dawley rats	5% or 10% flaxseed (equivalent to 3750 or 7500 mg/kg bw/day) or SDG at the equivalent of 5% flaxseed (1.5 mg/day)	Through pregnancy and lactation	No effect on pregnancy outcome except 10% flaxseed lowered birth weight and had hormonal effects: female offspring had shortened anogenital distance, increased uterus and ovary relative weights, earlier age and lower bodyweight at puberty, lengthened estrous cycle and persistent estrus; males had decreased postnatal weight gain and greater sex gland and prostate relative weights. However, 5% flaxseed tended to produce opposite effects. Results with SDG paralleled those with 5% flaxseed, suggesting that the effect is due to the lignan. CONCLUSION: 5 or 10% flaxseed or SDG produced no adverse effects on pregnancy other than lowered birth weights at 10% flaxseed, but there were some hormone-related effects on offspring that may affect reproduction.

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Table 32. Safety Studies in Animals

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Tou et al. 1999	Determine effects of flaxseed intake on reproductive development when given at different developmental stages.	Fed basal diet or basal diet supplemented with 5% or 10% flaxseed starting at gestation or at weaning and continuing to PND 132.	Sprague-Dowley rats	5% or 10% flaxseed dietary con-centration, equivalent to 3750 or 7500 mg/kg bw/day	From gestation or weaning to PND 132	Flaxseed exposure after weaning had no effect on the onset of puberty, but lifetime exposure had conflicting effects depending on the dose—5% delayed puberty and increased body weight at puberty, while 10% had opposite effects. Similarly, flaxseed exposure after weaning had no effect on the estrus cycle, but lifetime exposure at 10% lengthened the estrus cycle. The lifetime 10% flaxseed groups of both sexes had higher serum estradiol levels and the males had higher serum testosterone; there were no other effects. No effect was seen on any organs other than the sex organs. Both 5% and 10% flaxseed increased immature uterus weight, but not relative mature weight; 10% flaxseed also increased relative ovarian weight in females and relative sex accessory gland, seminal vesicle, prostate, and testes weights, as well as mild inhibition of prostate cell proliferation. Post-weaning flaxseed had no effect on males. CONCLUSION: Dose, timing, and duration of flaxseed exposure have important implications on reproduction.
Tou and Thompson. 1999	Determine effects of intake of flaxseed or secoisolariciresin ol diglycoside (SDG) on mammary-gland structures of female offspring when given at different developmental stages.	Fed basal diet or basal diet supplemented with 5% or 10% flaxseed or 1.82% flaxseed oil or SDG at the equivalent of 5% flaxseed to rats during pregnancy and lactation. At weaning, female offspring were assigned to 1 of the 4 diets.	Sprague-Dowley rats	5% or 10% flaxseed (equivalent to 3750 or 7500 mg/kg bw/day) or 1.82% flax-seed oil or 1.5 mg SDG (equivalent to the content of 5% flaxseed)	Prenatally and from weaning to PND50	Exposure to flaxseed after weaning had no effect. Flaxseed at 10% produced endocrine changes—early puberty onset, lengthened estrus cycles, and increased mammary gland differentiation (fewer terminal end buds and more alveolar buds). However, flaxseed at 5% resulted in delayed puberty onset and a reduced number of estrus cycles, leading to atrophy of mammary TEB structures. SDG at the equivalent level produced similar effects, but flaxseed oil did not. CONCLUSION: Exposure to 5% or 10% lfaxseed induced structural changes in the mammary gland that may potentially reduce mammary cancer risk.

000100

Table 32. Safety Studies in Animals

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Velasquez et al. 2003	Investigate the effects of different protein sources (casein, soy, or flaxseed) on renal function and nephropathy.	Feed obese rats 1 of 3 diets—20% casein, soy protein concentrate, or flaxseed meal.	Male obese SHR/N-cp rats	20% flaxseed meal dietary concentra-tion, equivalent to 15000 mg/kg bw/day	6 months	No differences between groups on feed intake or body weight gain, nor on plasma glucose levels. Plasma insulin was lower in the flaxseed meal group. Mean plasma creatinine, creatinine clearance, and urinary urea excretion did not differ, but urinary protein excretion was lower in the flaxseed groups. The rats in the flaxseed groups had the lowest incidence of abnormal glomeruli with mesangial expansion and the lowest tubulointerstitial scores. The authors concluded that onsumption of flaxseed meal exerts a renal protective effect with no indication of any adverse effects. The NOAEL was 20% dietary concentration, equivalent to 15,000 mg flaxseed/kg bw/day, the only dose tested.
Ward et al. 2001a	Determine effect on reproductive indices of feeding of flaxseed or purified SDG during and after suckling.	Feed rat dams either basal diet, diet with 10% flaxseed or diet with the equivalent in SDG from the start of lactation; after weaning, keep pups on diet or back to basal diet to PND 50 or PND 132.	Pregnant Sprague-Dawley rats and their pups	10% flaxseed dietary con-centration (equivalent to 7500 mg/kg bw/day); diet with equivalent SDG based on concen-tration of 1.772 mg/g in flaxseed	To dam from beginning of nursing and to pup after weaning to PND 50 or 132	Maternal feed consumption, body weight, and relative uterine and ovarian weights did not differ. There were not differences in weight gain or relative anogenital distance of the pups through lactation, and no differences in age or body weight at puberty. No differences were seen in the length of the estrous cycle, prostate and testes weights, or uterus and ovary weights. There were no histological effects on reproductive organs. The authors concluded that feeding a diet with 10% flaxseed or the equivalent lignan during lactation only or continuously during lactation and post weaning does not affect reproductive indices in male or female offspring. The NOAEL for flaxseed was 10% dietary concentration, equivalent to 7500 mg/kg bw/day.

000101

Table 32. Safety Studies in Animals

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Ward et al. 2001b	Assess the effect of exposure to flaxseed or SDG on bone strength of male rats	Feed rat dams either basal diet, diet with 10% flaxseed or diet with the equivalent in SDG from the start of lactation; after weaning, keep pups on diet or back to basal diet to PND 50 or PND 132.	Pregnant Sprague-Dawley rats and their pups	10% flaxseed dietary con-centration (equivalent to 7500 mg/kg bw/day); diet with equivalent SDG based on concen-tration of 1.772 mg/g in flaxseed	To dam from beginning of nursing and to pup after weaning to PND 50 or 132	At post-natal day 50, measures of bone strength were reduced among rats receiving the 10% flaxseed diet, but there were no differences in bone size, bone mineral concentration, or bone mineral density.. No effect was seen in animals receiving SDG. At day 132 there were no significant differences between any of the groups with regard to bone size, strength, or mineralization. The authors concluded that exposing male rats to a diet containing 10% flaxseed or an equivalent quantity of SDG either during lactation only or through to early adulthood is safe with respect to bone health. The NOAEL in this study was the single dose tested, 7500 mg flaxseed/kg bw/day

000102

Table 32. Safety Studies in Animals

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Wiesenfeld et al. 2003	Investigate the effects of ingestion of dietary flaxseed and of defatted flaxseed meal on feed consumption, feed efficiency, body weight, serum and tissue fatty acid profiles, cholesterol, triglycerides, vitamins A and E, and clinical chemistry parameters.	Animals were mated and randomly assigned to 1 of 5 feeding groups. 10 pregnant rats per group were euthanized at gestation day 20; any pups >10 were culled at postnatal day 4. Pups suckled to postnatal day 21-23, then fed respective diet.	Pregnant Sprague-Dawley rats and their pups	Control or 1 of 4 test diets: 13% or 26% flax meal (FM), or 20% or 40% flaxseed; equivalent to 9750 or 19,500 mg/kg bw/ day flaxseed meal and 15,000 or 30,000 mg/kg bw/ day flaxseed	From impregnation to gestation (c. 21-23 days) and until postnatal day 90	No effect was seen on body weight, absolute weights of liver, heart, spleen, or kidney; or growth of pregnant rats or F_1 rats; no effect on relative organ weights in F_1 rats. Relative heart weight was increased in both high FM and high FS groups. Histopathological examination revealed no treatment-related adverse effects. α-linolenic acid increased in serum and liver of pregnant and F_1 rats receiving FS. No effect on serum SFA of pregnant rats, but increase in MUFA (oleic acid) and EPA in high FS group and decrease in linoleic and arachidonic acid; no effect on DHA. In F_1 rats, decrease in linoleic and arachidonic acid and increase in EPA. Pregnant and F_1 rats receiving FS increased liver SFA (stearic) and EPA and decreased linoleic and arachidonic acid. Pregnant rats showed increase in behenic acid. Among stomach fatty acids, α-linolenic acid increased. 4-day-old pups whose dam received FS or FM had lower SFA (palmitic) and arachidonic acid and higher EPA; no effect on DHA. No significant effects were seen in liver or serum Prostaglandin E_2 in pregnant or F_1 rats. High FS group F_1 rats had decreased total cholesterol and HDL; no effect on serum triacylglycerols. Serum Ca was reduced in most FM and FS groups, but no effects on serum Fe, blood urea nitrogen, albumin, aspartate aminotransfer-ase, or alkaline phosphatase. Rats in the high FS group had increased alanine aminotransferase and reduced total protein and creatinine. FS significantly reduced liver, but not serum, levels of vitamin E. High FM reduced vitamin A. CONCLUSION: High levels of FS or FM did not adversely affect growth of pregnant or F_1 rats. α-linolenic acid from FS and FM was bioavailable and resulted in changes in C18 and C20 fatty acid metabolism. It is not clear if this in turn has adverse effects on oxidative stress or bone metabolism.

000103

Table 32. Safety Studies in Animals

Reference	Objective	Study Design	Animal Model	Daily Dose and Source	Duration of Feeding	Findings
Wolterbeek et al. 2004	Determine prenatal developmental toxicity of the lignan 7-HMR, a minor constituent of flaxseeds but differing only slightly from the major lignan metairesinol	Female Wistar rats were fed diets with 0, 0.25, 1, or 4% K-acetate complex of 7-HMR for days 0-21 of gestation	Mated female Wistar rats	0, 0.25, 1, or 4% potassium acetate complex of 7-HMR, approximately 0, 0.16, 0.60, and 2.06 g/kg bw/day	21 days (gestation days 0 to 21)	No effects were seen on reproductive performance nor were any treatment-related findings noted. However, half of the mated dams in the high-dose group failed to thrive due to large decreases in their feed intake and were sacrificed early, and body weights of the remaining members of the high-dose group were reduced. The NOAEL for maternal effects was the midrange concentration of 1% and the NOAEL for fetal development throughout gestation was the high concentration of 4%.
Yuan et al. 1999	Evaluate the antioxidant potential of dietary flaxseed in young female rats	Weanling female Sprague-Dawley rats (10/group) aged 24 days were divided into 3 treatment groups: 1) basal diet (control); 2) basal diet plus ground flaxseed; 3) basal diet with SDG	Weanling female Sprague-Dawley rats	10% ground flaxseed (about 7500 mg/kg bw/day) or 9 mg SDG/kg bw/day	50 days	Growth, feed efficiency, and liver-to-body weight ratios did not differ between treated and control groups. Total urinary lignans were significantly higher in flax-fed and SDG-fed rats compared to controls. Hepatic glutathione levels and glutathione peroxidase and catalase activity were similar between treated and control animals. No adverse effects were seen at an intake of SDG of 9 mg/kg bw/day or a flaxseed concentration of 10%, approximately corresponding to 7500 mg/kg bw/day of ground flaxseed. The NOAELs were 7500 mg flaxseed /kg bw/day and 9 mg SDG/kg bw/day, the only doses tested.

000104

4.3. Human Studies

The human studies of the effects of oral exposure to flaxseed or its constituents discussed in Section 4.3 are summarized in Table 34 at the end of the section.

Flaxseed has been fed to humans in a number of studies. None of these studies was designed to evaluate safety or tolerance, but measures bearing on these endpoints were included in some of them. It is notable that, as discussed in more detail below, in none of the large number of human studies presented below were any adverse effects reported; many of the study reports note specifically that such effects were not observed.

Prospective human studies are of particular value in identifying the functional properties of flaxseed that are observable over a short intervention period, while observational studies, such as cohort and case-control studies, are better suited for generating hypotheses regarding longer-term effects. Such studies are discussed in the following section as they pertain to the safety of increased exposure to α-linolenic acid (ALA).

In this section, a number of the studies are of specific use to assess the ability of flaxseed to provide those benefits named by Health Canada (1988) and the Macronutrients Panel of the Institute of Medicine (IOM 2001) as "established physiological effects" that are frequently characteristic of added fibers: attenuation of the postprandial blood glucose response, attenuation of blood cholesterol concentrations, and improved laxation. Note that neither Health Canada nor the IOM panel suggested that a substance needs to provide all three of these benefits in order to be regarded as an added fiber, nor did either exclude defining a substance as an added fiber based on an ability to confer benefits other than these. It may be noted that nearly all of the studies assessed the effects of whole flaxseed rather than specific components such as mucilage, ALA, or SDG; the rare exceptions are noted.

Since this GRAS determination addresses whole and milled flaxseed, and not its constituents, no effort is made below to determine which specific constituents of flaxseed may be responsible for effects observed during administration of whole or milled flaxseed.

This section is organized by the endpoint assessed in each study. Since some studies addressed more than a single endpoint, they are cited under each such endpoint, unavoidably resulting in some redundancy.

4.3.1. Physiological Effects of Flaxseed

4.3.1.1. Glycemic Response

Cunnane et al. (1993) conducted an intervention study in which 5 healthy men and 1 healthy woman (average age 30) consumed, in random order, a test meal containing 50 g of bread made from either wheat flour or flaxseed flour. They later consumed meals with either 50 g of glucose alone or 50 g of glucose mixed with 25 g of extracted flaxseed mucilage. Fingerprick blood samples were taken before the meal and at 15, 30, 45, and 60 minutes after the

beginning of consumption. The incremental area under the blood-glucose curve was 28% lower after consumption of flaxseed bread than after wheat bread. The addition of flaxseed mucilage reduced the area under the blood-glucose curve by 27%. The authors suggested that, "the increase in blood glucose caused by glucose-rich cereals or snack products could be potentially reduced if these products also contained flaxseed meal or mucilage. This could increase the range of foods available to diabetic patients." They concluded that, "From the point of view of safety, acceptability and efficacy, flaxseed appears to be a grain with useful potential in human nutrition."

In a second study, Cunnane et al. (1994) gave muffins providing either 0 or 50 g flaxseed/day to 10 healthy young adults (5 males and 5 females; mean age = 25 years) for 4 weeks, followed by a washout period, and then the other treatment in a randomized crossover design. For the men, the washout period was 2 weeks. For the women, the two treatment periods were synchronized to the same phase of their menstrual periods and so varied from 3 to 5 weeks. Blood was sampled at baseline and at the end of each feeding period; samples of subcutaneous fat were taken by suction needle biopsy from the anterior abdominal wall at the end of each feeding period. Study participants also took an oral glucose challenge one day before the end of each feeding period. The muffins were considered palatable and compliance was good. There was no significant difference on the oral glucose tolerance tests between groups in fasting blood glucose concentrations, peak blood glucose, or total area under the curve after 4 hours. However, glucose concentrations at 30 minutes were significantly lower after the flaxseed muffins were consumed for 4 weeks than after the control muffins were consumed for 4 weeks. Adipose tissue concentrations of α-linolenate were not significantly related to the area under the blood glucose curve. The authors concluded that consumption of flaxseed by healthy young adults improves laxation, reduces LDL cholesterol, and raises n-3 fatty acids in plasma and adipose tissue, and that these effects occur "in the absence of a significant deleterious effect on glucose tolerance, plasma peroxide concentrations, or antioxidant vitamin status."

In a randomized crossover trial, Lemay et al. (2002) compared the effects of flaxseed on glycemic response with those of hormone replacement therapy in hypercholesterolemic menopausal women. After a 4-month hypocholesterolemia diet, 25 menopausal women aged 45-65 years (mean = 58.3 years) with total cholesterol over 240 mg/dL were randomized to receive either 40 g/day of crushed flaxseed or to take hormone replacements, conjugated equine estrogens for those who had previous hysterectomy (n = 10) or micronized progesterone for those with intact uterus (n = 15). Treatment continued for 2 months, followed by a 2-month washout period, and then the alternate treatment for 2 months. The women visited the clinic at baseline and at months 2, 4, 5, 6, 7, 8, 9, and 10 and were evaluated for clinical symptoms, side effects, use of medications, weight, waist and hip girth, blood pressure, and dietary record, and blood was drawn for analysis of lipids, glucose, insulin, and sex hormone binding globulin. There was no difference in the two regimens in significantly reducing glucose and insulin levels. The authors concluded that, "the results of this 10-month cross-over study indicate that ... 40 g of crush flaxseed is as effective as 0.625 mg of conjugated equine estrogens ... in lowering serum levels of glucose and insulin ..."

In a companion experiment to one assessing the effect of flaxseed fiber on laxation, Dahl et al. (2005) investigated the effect of flaxseed on glycemic response using finely ground flaxseed meal (i.e., after extraction of oil) that comprised 56% soluble fiber and 44% insoluble fiber. Eleven healthy adults (including both males and females, but the numbers of each were not reported) aged 20-26 years (mean = 22.8 years) consumed a single serving of bread providing 50 g of available carbohydrate, either with added flaxseed meal or control. A typical portion of bread contained about 11 g flax fiber providing 2.7 g soluble fiber. Each volunteer participated in 4 sessions, 2 with each treatment. Capillary blood glucose levels were assessed at 0, 15, 30, 60, 90, and 120 minutes. The flaxseed meal treatment resulted in a significantly reduced area under the curve of glycemic response as well as a lower peak response. The authors observed that, "Despite this relatively small portion of soluble fiber, a significant reduction in glycemic response was observed. This suggests that the use of flax fiber provides sufficient soluble fiber to produce a delay in postprandial blood glucose elevation."

The evidence for a role of flaxseed in attenuating glycemic response is weak. Only 4 studies have addressed this endpoint. Findings have been generally supportive of the hypothesis of such an effect, but the magnitude of the effect has been quite small, particularly in view of the large doses (40 to 50 g/day) of flaxseed employed. Basch et al. (2007) evaluated the evidence supporting this endpoint as part of the Natural Standard Research Collaboration and assigned the level of evidence a grade of "C," meaning "unclear or conflicting scientific evidence."

4.3.1.2. Blood Lipids

Cunnane et al. (1993) conducted 2 experiments to investigate the nutritional aspects of flaxseeds in the human diet. Experiment 1 was intended to assess the bioavailability of the ALA in ground flaxseed. In a randomized crossover study, 5 healthy females (average age 20 years) consumed either flaxseed oil capsules or flaxseed flour for 4 weeks with a 2-week washout period in between. Both treatments provided 20 g/day of flaxseed oil containing 12 g/day of ALA. Changes in plasma ALA were equivalent, suggesting that the ALA in ground flaxseed is highly available. In Experiment 2, 9 healthy female college students (average age 24 years) consumed either a supplement of flaxseed flour (n = 5) or bread baked with 25% (w/w) flaxseed flour, both treatments providing 50 g flaxseed/day. After 4 weeks the treatments were discontinued during a 2-week washout period. There was no difference in the effects of flaxseed consumed as a supplement v. in bread; both treatments increased ALA and total long-chain n-3 fatty acids in both plasma and erythrocyte lipids. Both treatments also had no effect on triacylglycerol levels but reduced both total and LDL cholesterol. The authors concluded, "From the point of view of safety, acceptability and efficacy, flaxseed appears to be a grain with useful potential in human nutrition."

Bierenbaum et al. (1993) administered 15 g/day of ground flaxseed in bread for 3 months to 15 hyperlipidemic (serum cholesterola >240 mg/dl) but otherwise healthy adults (5 males and 10 females aged 21-65 years; mean age = 52.2 years) on vitamin-E therapy[1]. Total cholesterol

[1] The participants had just completed a 3-month trial of the effect of 800 IU/day of α-tocopherol; this study was continued with the addition of flaxseed.

and LDL were significantly reduced from baseline (i.e., from the end of the vitamin-E-supplementation study) by 18 and 19 mg/dl, respectively, while HDL and triacylglycerol were unchanged. Thrombin-stimulated platelet aggregation and lipid oxidation products were also signficantly decreased and no adverse effects were reported.

In a study discussed in the previous section, Cunnane et al. (1994) gave muffins providing either 0 or 50 g flaxseed/day to 10 healthy young adults (5 males and 5 females; mean age = 25 years) for 4 weeks. Blood was sampled at baseline and at the end of each feeding period. During flaxseed consumption, α-linolenate, eicosapentaenoate, and docosapentaenoate were significantly increased in plasma phospholipids and triacylglycerols; α-linolenate also increased in adipose tissue. LDL cholesterol was significantly decreased. The authors concluded that, "consumption of traditional flaxseed by healthy young adults ... modestly reduces plasma LDL cholesterol [and] raises n-3 polyunsaturates in plasma and adipose tissue."

Clark et al. (1995) studied the effect of flaxseed on lipid parameters and other endpoints in 9 lupus nephritis patients, 8 of whom completed the study (2 males and 6 females aged 32 to 72 years, mean age = 44.5 years; the age and sex of the drop-out patient, who withdrew due to scheduling problems, were not reported). Each patient consumed 15 g/day of milled flaxseed for 4 weeks, then 15 g twice a day (30 g/day) for the ensuing 4 weeks, and finally 15 g three times a day (45 g/day) for the next 4 weeks. This was followed by a 5-week washout period. Compliance, disease activity, blood pressure, plasma lipids, rheology, platelet activating factor (PAF)-induced platelet aggregation, renal function, and serum immunology were assessed. Good compliance was achieved for the 15 and 30 g doses, but 3 subjects had difficulty ingesting 45 g/day of flaxseed due to increased laxation. Blood pressure was unchanged throughout the study. Total and LDL cholesterol, and whole blood viscosity were significantly reduced with 30 g and 45 g doses; these effects persisted throughout the 5-week washout period. In general, the 30 g/day dose of flaxseed was well tolerated and conferred benefits in terms of improved creatinine clearance as well as atherogenic mechanisms important in the pathogenesis of lupus nephritis. Other than the increased laxation at the 45-g/day level, no adverse effects were reported. The authors concluded that, "30 g/day of flaxseed is well tolerated by patients with SLE [systemic lupus erythematosus] nephritis, and it exerts significant beneficial effects on renal function, plasma lipids, blood viscosity and complement C3 levels."

In a randomized double-blind crossover study, Arjmandi et al. (1998) compared the effects of flaxseed and sunflower seed on the lipid profile of postmenopausal women. During treatment periods lasting 6 weeks each, with a 2-week washout period, 38 otherwise healthy hypercholesterolemic postmenopausal women (mean age = 56.3 years) consumed 38 g/day of either flaxseed or sunflower seed in bread and muffins. Participants maintained a dietary record of daily intake, which was subjected to nutrient analysis. Anthropometric measures (height, weight, BMI, arm-muscle circumference, skin-fold thickness, and waist/hip ratio) and venous blood samples were obtained from all participants at the beginning and end of each treatment phase. Blood was analyzed for serum follicle stimulating hormone, 17β-estradiol, total cholesterol, HDL cholesterol, lipoprotein(a), and triacylglycerols. No anthropometric effects were observed. While both treatments significantly reduced total cholesterol from baseline, only the flaxseed significantly lowered LDL, as well as lipoprotein(a). Neither treatment significantly

affected HDL or triacylglycerol concentrations, and no adverse reactions were reported to either treatment. The authors summarized their findings as indicating that, "flaxseed, but not sunflower seed consumption, lowers serum LDL-cholesterol and Lp(a) concentrations despite excess energy intake." However, there were no anthropometric changes, and excess energy intake means little without knowing net change.

In a study assessing the effects of a test article other than whole flaxseed, Jenkins et al. (1999) evaluated the effect of partially defatted flaxseed on serum lipids, oxidative stress, and hormonal activity. In a randomized, single-blind, placebo-controlled crossover trial, 29 otherwise healthy hyperlipidemic subjects (22 males and 7 postmenopausal women aged 41-73 years; mean age = 57 years) consumed muffins that provided 50 g/day of partially defatted flaxseed with 20 g fiber/day or wheat bran (control) with the same fiber content for 3 weeks, separated by a 2-week washout period. Blood pressure and body weight were measured on day 0 and at the end of week 3 in both periods and blood samples were obtained and analyzed for total and HDL cholesterol and triacylglycerol concentrations, apolipoproteins A-1 and B, protein thiol groups and carbonyl groups, and agonistic and antagonistic activity of steroid hormones. Participants maintained diet records that were analyzed to assess compliance and estimate nutrient intake.

The enrollment included 37 subjects, but 8 withdrew from the study for reasons unrelated to the treatments (except for 1 subject who disliked the taste of the muffins containing flaxseed). Intakes of energy, carbohydrate, protein, and fat did not differ between the flaxseed and control phases. Significant reductions were seen in total and LDL cholesterol and apolipoprotein B during the flaxseed feeding period, along with a significant increase in triacylglycerols. Thiol concentrations were significantly lowered, which the authors suggested may indicate increased oxidative stress. No changes were seen in body weight, blood pressure, apolipoprotein A-1, protein carbonyl content, or agonistic or antagonistic activities of serum androgen and progestin. Since the lipid effects of partially defatted flaxseed were similar to those of full-fat flaxseed, Jenkins et al. (1999) suggested that these effects may be due to the mucilage content of the flaxseed.

In a randomized crossover trial, Clark et al. (2001) administered either 0 or 30 g/day of ground flaxseed (in 15-g sachets taken twice a day) to 23 patients (ages and sexes not reported) with lupus nephritis for 1 year. This was followed by a 12-week washout period and the reverse treatment for 1 year. Serum viscosity and plasma lipids were measured at baseline and at 6-month intervals. Compliance was assessed by counts of flaxseed sachets and serum phospholipids measures. Eight participants dropped out, with the major issue being difficulty in ingesting 30 g flaxseed/day, and only 9 of the 15 remaining subjects complied with the flaxseed diet. Two of the 15 reported increased laxation when taking flaxseed supplementation. Plasma lipids and serum viscosity were unaltered by the flaxseed supplementation. The few patients who complied with the flaxseed supplementation over the period of 1 year had improved remal function marked by significant reductions in serum creatinine. No adverse effects were reported, but the authors noted that, "30 grams of flaxseed, which were well tolerated in the 12-week dosing study, were not well tolerated over one year."

Tarpila et al. (2002) completed a controlled double-blind crossover study to assess the effects of flaxseed on serum lipids and enterolactone. Eighty food-company employees (62

women, 18 men, mean age 43 years) consumed either a control diet or a diet that contained 1.3% ground flaxseed and 5% flaxseed oil (along with 3-4% inulin and wheat fiber) for 4 weeks with a 4-week washout period[1]. Fifteen subjects continued an open trial with the flax-supplemented meals for 4 additional months. Blood parameters were measured at baseline, after both intervention periods, and at 2 and 4 months during the open study. The measures included hemoglobin, leukocytes, aspartate and alanine amino transferases, alkaline phosphatase, creatinine, total cholesterol, HDL and LDL cholesterol, triacylglycerols, total and individual fatty acids, and enterolactone. The participants consumed an average of 21.5 g flaxseed/day. No significant changes were seen in any of these measures except increased levels of enterolactone and altered concentrations of some of the individual fatty acids. Specifically, no differences were seen in total, HDL, or LDL cholesterol, in triacylglycerols, or in myristic, pentadecanoic, palmitic, palmitoleic, stearic, linoleic, arachidonic, or docosahexaenoic acids; levels were lowered of oleic, vaccenic, and dihomo-γ-linoleic acid; and increased levels existed of α- and γ-linolenic acids and their metabolites eicosapentaenoic and docosapentaenoic acids. The authors concluded that the addition of flaxseed products to the diet may produce beneficial changes in blood levels of enterolactone and α-linolenic acid without causing deleterious effects.

Lucas et al. (2002) conducted a randomized double-blind study to evaluate the effects of consuming 40 g flaxseed/day for 3 months compared to a placebo based on wheat flour on plasma lipids of healthy postmenopausal women younger than 65 years (mean age = 54.5 years). Excluded were women on hormone replacement therapy or with cancer, liver disease, hypothyroidism or hyperthyroidism, GI disorders, type I diabetes, pelvic inflammatory disease, or endometrial polyps. Fifty-eight women (29 per group) were randomized to receive ground whole flaxseed or placebo. A complete medical history was obtained and all women were given routine physical and gynecological examinations at baseline and at the end of the study. Overnight fasting blood was drawn at the beginning and end of the study and analyzed for total and HDL cholesterol, triacylglycerols, apolipoprotein A-1, apolipoprotein B, IGF-I, IGF-binding protein, 17β-estradiol, estrone, total alkaline phosphatase and tartrate-resistant acid phosphatase, calcium, and bone-specific AP activity. Twenty-four hour urine was also collected at baseline and termination.

Only 36 women completed the study, 20 from the flaxseed group and 16 from the placebo group. Withdrawal reasons potentially related to treatment included gastrointestinal problems (3 flaxseed, 6 placebo) and lack of palatability of the dietary regimen (6 flaxseed, 3 placebo). Flaxseed supplementation significantly lowered both total and non-high-density cholesterol and apolipoproteins A-1 and B; other measures were not significantly affected.

In a study discussed in the previous section, Lemay et al. (2002) also compared the effects on serum lipids of 40 g flaxseed/day with those of hormone replacement therapy in hypercholesterolemic menopausal women (n = 25; mean age = 58.3 years) in a randomized crossover trial with 2 months in each condition. Both regimens significantly reduced LDL

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[1] The diet foods, which could be either unsupplemented or prepared with flaxseed and flaxseed oil, included hamburger, chicken meat balls, pizza, sausage, lasagne, cold cuts, and similar foods.

cholesterol, but only the hormone-replacement therapy was effective in increasing HDL cholesterol and apolipoprotein A-1 and in lowering plasminogen activator inhibitor type 1.

Stuglin and Prasad (2005) investigated the effects of short-term flaxseed on blood pressure, serum lipids, and the hemopoietic system. Fifteen healthy males age 22-47 (mean age = 27.3 years) consumed 32.7 g flaxseed/day (in 3 muffins) for 4 weeks. Blood pressures, heart rates, and fasting blood samples were collected before and after the feeding period. Blood was analyzed for total, LDL, HDL, and VLDL cholesterol; triacylglycerols; protein; albumin; total bilirubin; AST; ALP; glucose; creatinine; urea, hemoglobin; reb blood cells; white blood cells; and neutrophils. Concentrations of triacylglycerols increased and those of creatinine decreased, but the remaining parameters remained unchanged: blood pressure, heart rate, and all blood measures. The authors concluded that, "flaxseed consumption [of 32.7 g flaxseed/day] for 4 weeks does not have deleterious effects on the liver, kidney, and hemopoietic system."

Dodin et al. (2005) randomly assigned 199 healthy menopausal women aged 45-65 years (mean age = 54.8 years) to consume either 40 g flaxseed/day (n = 101) or a wheat-germ placebo (n = 98) for 12 months. At the time of enrollment, weight, height, waist circumference, and blood pressure were measured; clinical and gynecological examinations were performed; demographic characteristics, gynecological history, and smoking and alcohol consumption were self-reported; dietary intake of phytoestrogens in the past month was evaluated with a food-frequency questionnaire; and a 3-day food diary was recorded. Sampling was conducted at baseline and month 12.

About 10% of women in the flaxseed group and 5% in the wheat-germ group dropped out due to digestive problems (specific problems not stated). Blood pressure was significantly reduced over the 12 months only in the flaxseed arm, but did not differ significantly from controls. Total and HDL cholesterol were significantly reduced in the flaxseed group but slightly elevated in the wheat-germ group; there was an insignificant reduction in LDL cholesterol and no change in triacylglycerols or bone mineral density (BMD). Aside from the digestive issues likely due to the high level of dietary fiber provided by 40 g flaxseed/day, no adverse side effects were observed. The authors noted that, "flaxseed incorporation into the diet compared with wheat germ placebo has favorable, but limited, effects on blood cholesterol and causes no change in BMD ... [and] had no clinically significant effect on weight gain, BMI, or blood pressure compared with wheat germ."

Hallund et al. (2006) studied the effects of 500 mg/day of SDG extracted from flaxseed on 22 postmenopausal women (mean age = 61 years) in a randomized double-blind placebo-controlled crossover study. Women consumed a low-fat muffin, with or without SDG, for 6 weeks, separated by a 6-week washout period. Body weight, blood pressure, and blood and urine were sampled at the beginning and end of each treatment period. Blood factors included enterolactone , total cholesterol, LDL, HDL, triacylglycerols, antioxidant capacity, ferric-reducing ability, and lipoprotein oxidation lag time; the urinalysis assessed enterolactone excretion. Compliance was excellent and minor gastrointestinal symptoms were reported by only 4 women during the SDG phase and 4 women during the placebo phase.. The SDG intervention (providing a dose of SDG equivalent to that provided by about 250 g flaxseed/day) increased

both plasma and urine levels of enterolactone; no other measures were significantly affected. No adverse effects attributable to the intervention were reported.

In a randomized double-blind placebo-controlled crossover study, Faintuch et al. (2007) administered 30 g/day of flaxseed flour or an equal mass of manioc flour (placebo) to 24 morbidly obese adults (4 male and 20 female, mean age = 40.8 years and mean BMI = 47.1 kg/m^2) outpatients. Each treatment period was 2 weeks with a 24-hour wash-out period. The test and placebo flours were given to the participants in 2 15-g bags, and participants were instructed to mix the contents with hot or cold drinks or with meals. A medical history was taken at baseline, and weight, height, and BMI were measured. Blood was analyzed for hemoglobin, white blood cell count, total cholesterol, HDL, LDL, VLDL, triacylglycerols, glucose, serum albumin, insulin, leptin, C-reactive protein, serum amyloid A, fibronectin, and complement fractions C3 and C4. Anthropometric and blood measures were taken at the end of each phase of the study. No intolerance was noted and all participants completed the study. There was no change in body weight nor any change in any of the biochemical indices. The investigators noted the absence of adverse effects on biochemical endpoints.

In summary, although there have been a large number of human studies addressing the effect of flaxseed consumption on blood lipids, the results are equivocal. Many studies have reported reductions in total cholesterol and LDL, but others have not. Generally the effects of consumption of even large doses (30 to 50 g/day) of flaxseed on blood lipids appear to be small. The meta-analysis conducted by Basch et al. (2007) reached a similar conclusion, assigning a grade of "C" (unclear or conflicting) to the evidence supporting this effect. While a beneficial effect of flaxseed consumption on blood lipids is not excluded, it has not been demonstrated to an acceptable level of certainty.

4.3.1.3. Laxation

Cunnane et al. (1994), in a study discussed in both of the preceding sections, gave muffins providing either 0 or 50 g flaxseed/day to 10 healthy young adults (5 males and 5 females; mean age = 25 years) for 4 weeks. The muffins were considered palatable and compliance was good. The flaxseed was mildly laxative, with bowel movements increasing 30% in frequency.

In a study discussed in the previous section, Clark et al. (1995) studied the effect of flaxseed on immune, inflammatory, rheologic, platelet, lipid, and renal clearance parameters in 8 lupus nephritis patients (2 males and 6 females aged 32 to 72 years), but also observed a laxation effect. Each patient consumed 15 g/day of milled flaxseed for 4 weeks, then 15 g twice a day (30 g/day) for the ensuing 4 weeks, and finally 15 g three times a day (45 g/day) for the next 4 weeks. Three subjects had difficulty ingesting 45 g/day of flaxseed due to increased laxation. In general, the 30 g/day dose of flaxseed was well tolerated and no adverse effects were reported.

In addition to studying glycemic response as discussed in Section 4.3.1.1, Dahl et al. (2005) performed a second experiment to investigate the effect of flaxseed on laxation using finely ground flaxseed meal (i.e., after extraction of oil) that comprised 56% soluble fiber and 44% insoluble fiber. Twenty-six healthy adults (7 males and 19 females aged 18-60 years)

established a 3-week baseline of stool collection, then were randomly assigned to consume either flaxseed meal or psyllium for 3 days, to increase the dose daily to 15 g/day by the end of a week, and to remain at that level to the end of a second week. All bowel movements were collected for the full 5-week period. Both fiber treatments significantly increased fecal mass over baseline; there was no difference between treatments.

In summary, 3 small studies to date have addressed the effect of flaxseed on laxation. The Natural Standard Research Collaboration paper by Basch et al. (2007) rated this evidence base as a "B," or "good scientific evidence." However, while the findings of the 3 studies have been consistent in showing an effect, the evidence base must be regarded as weak since it is based on studies with a total of 44 participants, only 31 of whom actually consumed flaxseed. Further, 8 of these individuals were patients with lupus nephritis, and consequently the laxation effect of flaxseed has been tested in only 23 healthy individuals.

4.3.2. Other Effects of Flaxseed

4.3.2.1. Hormonal Effects

A number of studies have investigated the effects of flaxseed or SDG on various hormonal endpoints, including male and female sex hormones.

To investigate the effects of flaxseeds on male sex hormones, Shultz et al. (1991) gave 6 healthy males aged 25-42 years (mean = 33.8 years) whole wheat/flaxseed bread for 6 weeks, providing 13.5 g/day of flaxseed flour. Compliance was assessed by daily tracking and by completion of 3-day diet records. Participants were weighed weekly. No changes were observed in mean body weight or BMI. Analyses of 24-hour urine specimens at baseline; after weeks 1, 2, 4, and 6; and at 2 weeks after cessation of feeding found enterodiol and enterolactone significantly raised by a factor of 7-28-fold over baseline; enterolactone excretion was 5-10-fold greater than enterodiol excretion. Fasting blood samples taken at baseline and following the 6-week flaxseed feeding showed no significant changes in plasma total testosterone, free testosterone, or sex-hormone-binding globulin. There were also no significant effects on liver function, plasma glucose, blood urea nitrogen, creatinine, alkaline phosphatase, calcium, phosphorus, sodium, potassium, chloride, triacylglycerols, or total cholesterol.

Phipps et al. (1993) evaluated the effect of 10 g/day of flaxseed powder on menstrual cycles. Eighteen women aged 20-34 years, healthy and with normal menstrual cycles, completed the study through 7 menstrual cycles; half took the flaxseed supplement during cycles 2-4 and the other half during cycles 5-7. Urinary samples were collected on cycle days 7-9 and blood was drawn 5 times per cycle beginning with cycle 2. Urinalysis measured enterolactone and enterodiol; blood was analyzed for estradiol, estrone, testosterone, progesterone, dehydroepiandrosterone sulfate, and sex hormone-binding globulin.

No anovulatory cycles occurred during the 36 flax cycles, compared to 3 occurrences during the 36 control cycles. The flaxseed cycles were consistently associated with significantly longer luteal phase (12.6 days v. 11.4 days), but there were no differences in blood hormone concentrations during flaxseed and control cycles. Urinary excretion of enterodiol and

enterolactone increased significantly (by 55-fold and 13-fold, respectively) during flaxseed cycles. The authors regarded the increased luteal-phase length and reduced anovulatory cycles induced by flaxseed ingestion as indicating a decreased risk of breast cancer. No adverse events were observed. The authors concluded that mammalian lignans produced by metabolism of flaxseed lignans have a role in the relationship between diet and sex steroid action and possibly between diet and hormonally dependent cancer.

In a study of the nutritional effects of flaxseed that was discussed in each of the previous sections, Cunnane et al. (1994) gave muffins providing either 0 or 50 g flaxseed/day to 10 healthy young adults (5 males and 5 females with mean age = 25 years) for 4 weeks in a randomized crossover design. For the men, the washout period was 2 weeks. For the women, the two treatment periods were synchronized to the same phase of their menstrual periods and so varied from 3 to 5 weeks. Blood, urine, and feces were sampled at baseline and at the end of each feeding period. The flaxseed muffins did not differ from the control muffins in thiobarbituric acid-reactive substances, but during flaxseed consumption urinary lignan excretion was increased more than 5-fold.

Lampe et al. (1994) conducted a balanced randomized crossover study in which 18 premenopausal women age 20-34 (mean age = 26.9 years) consumed their usual diets with or without supplementation with 10 g/day of ground flaxseed through 3 complete menstrual cycles, with no washout period between the phases. Women were excluded from the study if they used oral contraceptives or reported irregular menstrual cycles. (Thirty women were enrolled in the study; 7 withdrew before treatment began and 5 more women withdrew during treatment; reasons for withdrawal were not reported.) During the flaxseed phase, the women received the flaxseed packaged in 10-g sachets; they were instructed to split the contents dose into 2 or 3 servings and add it raw to prepared foods. Urine was collected during both the follicular and luteal phases of the menstrual cycles and analyzed for the lignans enterolactone, enterodiol, and matairesinol and the isoflavonoids daidzein, equol, genistein, and *O*-demethylangolensin. There were no differences between treatments in intakes of energy, carbohydrates, protein, or fat, but fiber intake was higher during the flaxseed phase. Consumption of flaxseed resulted in increased urinary excretion of enterolactone, enterodiol, and total lignan, but not in any change in excretion of matairesinol or any of the isoflavonoids tested. No differences were noted between urine samples collected in the luteal v. follicular phases, although the authors speculated that this was due to lack of adequate power in the study.

In a randomized crossover design (part of a larger study (Lampe et al. 1994) of the effects of flaxseed on lignan excretion and menstrual cycle), 13 healthy premenopausal women (mean age = 27.8 years) consumed either their regular diet or their regular diet supplemented with 10 g/day of ground flaxseed for three complete menstrual cycles (Kurzer et al. 1995). Women were excluded from the study if they used oral contraceptives, had unstable body weight, or had irregular menstrual cycles. There was no washout period between the 2 phases. During the flaxseed phase, the women were instructed to split their daily flaxseed dose into 2 or 3 servings and add it raw to prepared foods. A dietary record was maintained and used to estimate nutrient intake. Feces were collected on Days 7-11 of the last menstrual cycle in each diet period and analyzed for lignans and isoflavonoids. No effects were seen in consumption of energy,

carbohydrate, protein, or fat, and there were no changes in body weight or BMI. Flaxseed consumption significantly increased fecal excretion of the lignans enterodiol, enterolactone, and matairesinol, but there was no effect on fecal excretion of the isoflavonoids daidzein, equol, genistein, and *O*-demethylangolensin.

Jenkins et al. (1999; discussed in Section 4.3.1.2. Blood Lipids) evaluated the effect of partially defatted flaxseed on hormonal activity as well as serum lipids and oxidative stress. In a randomized placebo-controlled crossover trial with two 3-week treatment periods separated by a 2-week washout, 29 hyperlipidemic subjects (22 males and 7 postmenopausal women aged 41-73 years; mean age = 57 years) consumed muffins that provided 50 g/day of partially defatted flaxseed containing 20 g fiber/day or wheat bran (control) with the same fiber content. A dietary record was maintained and analyzed to measure compliance and estimate nutrient intakes. At baseline and at the end of week 3 in both phases, blood samples were obtained and analyzed for agonistic and antagonistic activity of steroid hormones measured by gene regulation of steroid receptors in isolated breast cancer cell lines. Intakes of energy, carbohydrate, protein, and fat did not differ between the flaxseed and control phases. No significant differences were seen in the agonistic and antagonistic activities of serum androgen or progestin between the test and control groups at either baseline or at the end of week 3.

In a randomized crossover design, Haggans et al. (1999) provided 0, 5, or 10 g/day of ground flaxseed to 28 healthy non-smoking postmenopausal women aged 53-82 years for 7-week periods with 7-week washout periods intervening. The ground flaxseed was prepared weekly by grinding commercially available whole flaxseed and was provided in tubes; the study participants consumed it at breakfast. During the last week of each feeding period the women provided 24-hour urine collections for 2 consecutive days. Urinary excretion of the estrogen metabolite 2-hydroxyestrogen increased, while that of 16α-hydroxyestrone did not. The authors concluded that flaxseed may have chemoprotective effects in postmenopausal women; the flaxseed was well tolerated and no adverse effects were reported.

Haggans et al. (2000) supplemented the habitual diets of 16 healthy premenopausal women aged 18-38 years (mean age = 26.7 years) with 1 of 4 treatments for 2 menstrual cycles each in a randomized crossover design. Each treatment began on day 1 (menses) and continued through 2 cycles; there were no washout periods. The 4 supplements were: nothing, 10 g/day of flaxseed, 28 g/day of wheat bran, and 10 g flaxseed + 28 g wheat bran/day. The flaxseed, wheatbran, or placebo was presented in 2 baked goods, muffins or cookies, to be consumed each day. Dietary records were maintained and analyzed for nutrient intakes, and body weights were measured. Urine samples were collected during the mid-luteal phase of the second menstrual cycle of each treatment period and analyzed for the estrogen metabolites 2-hydroxyestrogen and 16α-hydroxyestrone. There were no significant differences in carbohydrate or protein intake during any of the feeding phases, but fat intakes and thus total energy intakes differed, being significantly higher in the flaxseed + wheat bran group. Fiber intake was also significantly higher in the groups receiving wheat bran, especially that group receiving both flaxseed and wheat bran. Nevertheless, neither body weights nor BMIs differed significantly among the groups. Flaxseed, but not wheat bran, significantly increased excretion of 2-hydroxyestrogen and the ratio of the

two metabolites (2-hydroxyestrogen/16α-hydroxyestrone), regarded as chemoprotective against breast cancer risk.

Employing a randomized crossover trial, Hutchins et al. (2000) assessed the effects on ingestion of 0, 5, or 10 g/day of flaxseed on the lignan excretion of 31 healthy postmenopausal women aged 52-82 years (mean = 66.9 years). Women were excluded if they had GI disorders or food allergies, if they had used hormone replacement therapy, or if they consumed for than 2 alcoholic drinks a day. Each feeding period lasted for 7 weeks, with a 7-week washout period between the first and second feeding periods and a 14-week washout period between the last two feeding periods. During the flaxseed phases, women were given tubes containing 5 or 10 g of ground flaxseed; the flaxseed was typically consumed in a single serving at breakfast. Body weight was monitored, and the women completed 3-day diet records to monitor food intake. Urine samples collected for 2 days at the end of each feeding period were analyzed for content of the lignans enterodiol, enterolactone, and matairesinol.

Three of the 34 women originally enrolled withdrew for non-study-related reasons. Body weights and BMIs were unchanged. There were no significant differences between the phases in total energy, carbohydrate, protein, fat, or total fiber intakes. Excretion of matairesinol was not affected by flaxseed consumption, but excretion of enterolactone and enterodiol were significantly increased in a dose-dependent manner.

In a previously discussed study (Section 4.3.1.2. Blood Lipids), Lucas et al. (2002) conducted a randomized double-blind study to evaluate the effects of daily consumption of 40 g flaxseed or a comparative wheat-based control for 3 months on postmenopausal women under the age of 65 years. Food frequency questionnaires were completed at baseline and at the end of the study and analyzed for nutrient intakes. Physical and gynecological examinations were performed at the beginning and end of the study, urine samples were taken for analysis of creatinine and helical peptide, and overnight fasting blood was drawn and analyzed for 17β-estradiol and estrone activities.

Although 58 women were enrolled and 29 were randomly assigned to the test and control groups, only 36 women (20 receiving flaxseed and 16 controls) with a mean age of 54.4 years completed the study due to gastrointestinal problems with the wheat-flour placebo or palatability issues with flaxseed. No significant changes or differences between groups were seen in body weight or BMI, nor in nutrient intakes. The authors concluded that, "Flaxseed did not exert any estrogenic properties as assessed by unaltered circulating levels of sex hormones, hormone binding globulin, or maturation index.."

As discussed previously, in a randomized crossover trial Lemay et al. (2002) compared the effects of flaxseed on menopausal symptoms with those of hormone replacement therapy in 25 hypercholesterolemic but otherwise healthy menopausal women (mean age = 58.3 years). Women were randomized to receive either 40 g/day of crushed flaxseed or hormone replacements for 2 months, with an intervening 2-month washout period. There was no difference in the two regimens regarding decreasing menopausal symptoms, but only the hormone-replacement therapy was effective in increasing sex hormone binding globulin.

Tarpila et al. (2002) completed a controlled double-blind crossover study to assess the effects of flaxseed on enterolactone as well as blood chemistry and serum lipids. Lipids were discussed previously and blood chemistry is discussed below. Eighty food-company employees (18 men with a mean age of 45.6 years and 62 women with a mean age of 42.6 years) consumed either a control diet (described in Section 4.3.1.2) or a diet that contained 1.3% ground flaxseed and 5% flaxseed oil (along with 3-4% inulin and wheat fiber) for 4 weeks. Fifteen subjects continued an open trial with the flax-supplemented meals, providing 21.5 g flaxseed/day, for 4 additional months. Blood enterolactone was measured at baseline, after both intervention periods, and at 2 and 4 months during the open study. Levels of enterolactone were significantly increased for those consuming flaxseed. The authors concluded that the addition of flaxseed products to the diet may produce beneficial changes in blood levels of enterolactone and α-linolenic acid without causing deleterious effects.

Frische et al. (2003) supplemented the usual diets of 16 healthy premenopausal women aged 20-38 years with one of four treatments for 2 menstrual cycles in a randomized single crossover design. Each treatment began on day 1 (menses) and continued through 2 cycles; there were no washout periods. The 4 supplements were: nothing, 10 g/day of flaxseed, 28 g/day of wheat bran, and 10 g flaxseed + 28 g wheat bran/day. Blood samples were collected during the mid-luteal phase of the second menstrual cycle of each treatment period and analyzed for serum hormones (17β-estradiol, dehydroepiandrosterone sulfate, estrone, and progesterone) and sex hormone binding globulin. Urine samples collected at the same time were tested for lignan excretion. Lignan excretion increased during periods of flaxseed consumption, but there were no changes from any treatment on serum hormones or sex hormone binding globulin. The authors concluded that the chemoprotective effects reported for flaxseed may result from a mechanism other than a hormonal effect.

Brooks et al. (2004) compared the effects of consuming equal amounts of flaxseed or soy on estrogen metabolism in 46 postmenopausal women (mean age = 53.1 years). In a randomized, double-blind, parallel, placebo-controlled design, the habitual diets of the women were supplemented with either a whole-wheat flour placebo ($n = 15$), 25 g/day of soy flour ($n = 15$), or 25 g/day of ground flaxseed ($n = 16$) in a muffin consumed once a day for 16 weeks. The flaxseed provided 50 mg/day of SDG. Blood and 24-hour urine samples were collected at baseline and at the end of the 16 weeks. Urine samples were analyzed for phytoestrogens, the estrogen metabolites 2-hydroxyestrone and 16α-hydroxyestrone, and the serum hormones estradiol, estrone, and estrone sulfate.

The only dropouts from the study were 2 women in the soy-flour group. Urinary concentrations of 2-hydroxyestrone, but not of 16α-hydroxyestrone, increased significantly ($p < 0.05$) in the flaxseed group. In the flaxseed group, the ratio of 2-hydroxyestrone to 16α-hydroxyestrone was positively correlated with urinary lignan excretion ($r = 0.579$, $p < 0.02$), while no significant correlation was observed in the soy and placebo groups. Brooks et al. (2004) concluded that supplementation with ground flaxseed modifies urinary estrogen metabolite excretion to a greater extent than does supplementation with soy flour. This modification is associated with an increase in urinary lignan excretion. While the researchers hypothesized that

the effects observed were due to SDG, they conceded that the alpha-linolenic acid content of the flaxseed may have played a part. No adverse effects were reported in this study.

Dodin et al. (2005), in a study discussed earlier with regard to blood lipid effects, randomly assigned 101 healthy menopausal women aged 45-65 years to consume 40 g flaxseed/day for 12 months while a similar group of 99 women consumed a wheat-germ placebo. Menopausal symptoms were assessed at baseline and at month 12. About 10% of women in the flaxseed group and 5% in the wheat-germ group dropped out due to digestive problems. Both treatments reduced such menopausal symptoms as hot flashes and night sweats. Aside from the digestive issues likely due to the high level of dietary fiber provided by 40 g flaxseed/day, no adverse side effects were observed.

In a double-blind, randomized, controlled trial, Lewis et al. (2006) compared the effects of daily ingestion of soy flour, wheat flour, and ground flaxseed on quality of life and hot-flash frequency and severity in postmenopausal women. A total of 87 women, 1-8 years post-menopause, daily consumed muffins with 25 g of flaxseed providing about 50 mg of lignan (n=28), 25 g of soy providing about 42 mg of isoflavones (n=31), or wheat control (n=28) for 16 weeks. Hot flashes were significantly less severe with flaxseed than with the control muffins, but there was no significant effect on the quality-of-life instrument.

Hallund et al. (2006) studied the hormonal effects (as well as effects on blood lipids, discussed previously) of 500 mg/day of SDG extracted from flaxseed on 22 healthy postmenopausal women (mean age = 61 years) in a randomized double-blind crossover study. Women consumed low-fat muffins with or without SDG for 6 weeks. Blood and urine were sampled at the beginning and end of each treatment period. Blood factors included enterolactone and the urinalysis assessed enterolactone excretion. The SDG intervention increased both plasma and urine levels of enterolactone; no other measures were significantly affected. No adverse effects attributable to the intervention were reported.

In a randomized double-blind placebo-controlled crossover study, Faintuch et al. (2007) administered 30 g/day of flaxseed flour or an equal mass of manioc flour (placebo) to 24 morbidly obese (average BMI = 47.1) outpatients (4 males and 20 females, mean age 40.8 years). Each treatment period was 2 weeks with a 24-hour wash-out period. Neither treatment resulted in any change in biochemical indices, as discussed in Section 4.3.1.2, or in leptin activity.

In summarizing the conclusions of the 18 studies discussed above, in may be noted that only a few studies have been done on hormonal effects of flaxseed in males; one study assessed effects on testosterone and one study evaluated androgen and progestin; no effects were found. Two studies that investigated lignans found increases in both enterodiol and enterolactone excretion in males. A larger number of the studies discussed involved women, both menstruating and post-menopausal. These studies have consistently found increased levels of the lignans enterodiol and enterolactone in both groups, as well as increased levels of 2-hydroxyestrogen, an estrogen metabolite believed to be protective of breast cancer. One study also found increased excretion of matairesinol, but 2 others did not. One study found that administration of SDG alone boosted excretion of enterolactone. The studies discussed above generally found significant

changes in serum hormones (estradiol, estrone, progesterone) or sex hormone binding globulin. Finally, several studies of menopausal women found decreased symptoms such as hot flashes.

It is clear that consumption of flaxseed leads to the formation and eventual excretion of the lignans enterodiol and enterolactone, but significant hormonal effects have generally not been observed in either males or females, even at doses as high as 50 g flaxseed/day.

4.3.2.2. Cancer Progression

Demark-Wahnefried et al. (2001) reported on a pilot study of the effect of flaxseed supplementation in men with prostate cancer. Twenty-five otherwise-healthy prostate-cancer patients (mean age = 63.8 years) awaiting prostatectomy were placed on a low-fat (20% of energy or less) diet supplemented with 30 g/day ground flaxseed for the 21 to 77 days (average = 34 days) before surgery; only historical controls were available. At baseline and follow-up, levels of prostate-specific antigen (PSA), testosterone, free androgen index, and total serum cholesterol were measured, and tumor proliferation and apoptosis were compared with historical data. Total cholesterol was significantly lowered, as were total testosterone and free androgen index. Levels of PSA were unaffected. As compared with historical controls, both proliferation rate and apoptosis were favorably affected in a time-in-treatment-dependent manner. The only reported side effects were increased bowel movements (n=2), constipation (n=1), and flatulence (n=3); no nausea was noted.

In a randomized, double-blind, placebo-controlled study, Thompson et al. (2005) evaluated the effect of 25 g flaxseed/day on tumor biological markers in postmenopausal breast cancer. Nineteen women (average age 70 years) with newly diagnosed breast tumors were given flaxseed-containing muffins while awaiting surgery while 13 controls (average age 65 years) received placebo muffins made with whole-wheat flour. The women remained on their assigned treatments until surgery, an average of 32 days in the flaxseed group and 39 days in the control group. Tumor growth kinetics were examined based on tumor cell proliferation, apoptosis, c-erbB2 antibody expression, and estrogen and progesterone receptor levels. Urine samples were obtained and analyzed for lignan excretion. The flaxseed group had higher lignan excretion, and exhibited significant reductions in tumor cell proliferation and c-erbB2 expression and an increased level of apoptosis. The research team concluded that, "daily intake of 25 g flaxseed can significantly reduce cell proliferation, increase apoptosis, and affect cell signaling by reducing c-erbB2 expression of human breast cancer cells," and further, that flaxseed's "excellent tolerability ... may make flaxseed particularly attractive for studies in breast cancer prevention, where healthy women should be offered well-tolerated interventions for long-term use."

In a poster session, Snyder et al. (2007) summarized a study of the effects of flaxseed supplementation and dietary fat restriction on the composition of prostatic tissue in 161 prostate cancer patients (age and health status other than the prostate cancer were not reported) with prostatectomy surgery scheduled for at least 3 weeks later. Patients were assigned to 1 of 4 diets: 30 g flaxseed/day, dietary fat restriction to <20% of total energy, flaxseed supplementation + dietary fat restriction, or a control diet not further described. At baseline and follow-up, erythrocytes were analyzed for fatty acids. After surgery, prostatic tissue was analyzed for fatty acids. Men were on protocol for an average of 30-31 days. The men receiving flaxseed had

higher intakes of ALA and higher n-3/n-6 ratios than the other groups, and had higher levels of EPA in erythrocytes and prostatic tissue as well as higher n-3/n-6 ratios in prostatic tissue. The authors concluded that conversion of ALA to EPA occurs with flaxseed and may support its use in the treatment of prostate cancer.

While the results of the 3 studies presented above were all regarded as favorable, any beneficial effect of flaxseed consumption on the development or progression of either breast or prostate cancer must be regarded as tentative.

4.3.2.3. Bone Metabolism

Lucas et al. (2002) conducted a randomized double-blind study to evaluate the effects of consuming 40 g flaxseed/day for 3 months, as compared to a placebo based on wheat flour, on bone metabolism of 58 healthy postmenopausal women under 60 years of age who consumed their habitual diets. (Effects on blood lipids and hormones were also assessed, and these aspects of the study were previously discussed in the appropriate sections.) Physical and gynecological examinations were performed at the beginning and end of the study and blood was drawn. Blood was analyzed for calcium and bone-specific AP activity among other things. Flaxseed had no effect on bone metabolism. The authors concluded that, "The findings of this studyindicate that flaxseed supplementation improves lipid profiles but has no effect on biomarkers of bone metabolism in postmenopausal women."

Brooks et al. (2004), in addition to the assessment of hormonal effects discussed earlier, compared the effects of consuming 25 g/day ground flaxseed or soy for 16 weeks on biochemical markers of bone metabolism in 46 healthy postmenopausal women (mean age = 53.1 years) in a randomized, double-blind, parallel, placebo-controlled design. The women consumed their habitual diets supplemented with flaxseed, soy, or a whole-wheat placebo. The flaxseed provided 50 mg/day of SDG. Blood and 24-hour urine samples were collected at baseline and at the end of the 16 weeks and analyzed for biochemical markers of bone metabolism. No significant change in markers of bone metabolism was observed within or between the treatment groups. Brooks et al. (2004) concluded that supplementation with ground flaxseed is associated with an increase in urinary lignan excretion, but has no effect on bone cell metabolism. No adverse effects were reported in this study.

In a study discussed previously with regard to blood lipids and hormonal effects, Dodin et al. (2005) randomly assigned 101 healthy menopausal women aged 45-65 years to consume 40 g flaxseed/day for 12 months while a similar group of 99 women consumed a wheat-germ placebo. Both groups of women consumed their habitual diets. Bone mineral density (BMD) was assessed at baseline and at month 12. Neither the flaxseed nor the wheat germ was able to suppress the progressive bone loss observed in menopausal women.

Griel et al. (2007) studied the effect of n-3 fatty acids on bone health. Twenty-three mildly overweight and hypercholesterolemic adults (20 males and 3 females; mean age = 49.9 years) consumed 3 diets differing in their fat composition in a randomized, double-blind, balanced order, 3-period crossover design. Each diet period lasted 6 weeks separated by 3-week washout periods. The 3 diets were an "average American" diet relatively high in saturated fatty

acids and low in polyunsaturated fatty acids, a high-linoleic acid diet (including walnuts and walnut oil), and a high ALA diet (including walnuts, walnut oil, and 20 g flaxseed/day). All subjects completed the study with excellent dietary compliance. Bone health was assessed by serum concentrations of N-teleopeptides and bone-specific alkaline phosphatase, both markers of bone turnover. The ALA diet significantly reduced N-teleopeptides compared with the "average American" diet while the linoleic-acid diet was intermediate. There was no effect on bone-specific alkaline phosphatase levels, indicating that rates of bone formation were unchanged, but bone resorption was decreased. The authors concluded that the results suggest that, "incorporating plant sources of n-3 PUFA into the diet may provide health benefits not only to the cardiovascular system, but also to the skeletal system."

Although the study by Griel et al. (2007) found a significant decrease in bone resorption with a high ALA diet, three previous studies of the effects of flaxseed consumed for as long as 12 months on bone metabolism did not reveal any beneficial effect. It appears that the hypothesis that consumption of flaxseed offers any significant benefit on bone metabolism must be regarded as unproved.

4.3.2.4. Immune Function

Kelley et al. (1991), in a randomized crossover design, fed 10 healthy males age 21-37 years either basal diet or basal diet + 31.7 g/day flaxseed oil for 56 days[1]. While there was a 14-day stabilization period at the beginning, there was no washout period between treatments. The flaxseed oil supplement suppressed the proliferation of peripheral blood mononuclear cells cultured with T-cell mitogen, but concentrations of granulocytes, lymphocytes, and monocytes did not differ, nor was there any change in numbers of circulating T cells (Leu-4, CD3), helper-inducer cells (Leu 3a, CD4), suppressor/cytotoxic cells (Leu-2a, CD8), or B cells (Leu-12, CD19). Similarly, no differences were seen in concentrations of IgG. The authors concluded that the flaxseed oil tended to suppress some indices of cell-mediated immune function without affecting any markers of humoral immunity. No overt signs of health risks were observed in this study.

Clark et al. (1995) studied the effect of flaxseed on immune, inflammatory, rheologic, platelet, and lipid parameters in systemic lupus erythematosus (SLE) patients. Reported effects on blood lipids and on laxation were discussed previously. Eight patients (2 males and 6 females aged 32 to 72 years, mean = 44.5 years) consumed 15 g/day milled flaxseed for 4 weeks, then 15 g twice a day (30 g/day) for the ensuing 4 weeks, and finally 15 g three times a day (45 g/day) for the next 4 weeks, followed by a 5-week washout period. Measures of serum immunology were assessed. No significant alterations were observed with flaxseed dosing in total T helper (CD3+/CD4+), helper inducer (CD4+/CD29+), suppressor inducer (CD4+/CD45RA+) subsets, natural killer cells (CD3-/CD16+ and/or CD56+) or cytotoxic T cells (CD3+/CD16+ and/or CD56+). CD11b expression on neutrophils, a measure of C3bi receptors, was significantly

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[1] Meals were prepared at the USDA Western Human Nutrition Research Center on a 7-day rotating menu. The daily diets provided 3000 kcal, including 126 g protein, 425 g carbohydrates, and 87 g fat, along with 4 g crude fiber.

reduced with the 30 g dose. The authors concluded that, "30 g/day of flaxseed is well tolerated by patients with SLE nephritis, and it exerts significant effects on renal function, plasma lipids, blood viscosity and complement C3 levels."

In a randomized double-blind placebo-controlled crossover study discussed earlier (Section 4.3.2.1), Faintuch et al. (2007) administered 30 g/day of flaxseed or manioc flour (placebo) to 24 morbidly obese outpatients, 4 males and 20 females with a mean age of 40.8 years. Each treatment period was 2 weeks with a 24-hour wash-out period. Flaxseed significantly reduced the inflammatory markers C-reactive protein, serum amyloid A, and fibronectin, but not complement fraction C3, or complement fraction C4. The investigators attributed the immune-modulatory effect to the ALA content of the flaxseed.

The three available studies of the effect of flaxseed consumption on immune factors are consistent in showing a moderate immunosuppressive effect. One experimenter attributed this effect to the ALA content of flaxseed. In light of the known anti-inflammatory effect of fish oil high in EPA and DHA (see, e.g., IOM 2002), this is not an unexpected finding or an unreasonable suggestion. The IOM panel noted that EPA and DHA are biologically more potent then their precursor, ALA. While declining to set a UL for EPA and DHA, the panel warned against overuse of supplements containing them; however, little research has been conducted with ALA and the panel apparently saw no need for caution regarding consumption of foods high in this n-3 fatty acid (IOM 2002).

4.3.2.5. Blood Chemistry

Shultz et al. (1991) gave 6 healthy males age 25-42 whole wheat/flaxseed bread for 6 weeks, providing 13.5 g/day of flaxseed flour. As discussed in Section 4.3.2.1, fasting blood samples showed no significant changes in plasma total testosterone, free testosterone, or sex-hormone-binding globulin. There were also no significant effects on liver function, plasma glucose, blood urea nitrogen, creatinine, alkaline phosphatase, calcium, phosphorus, sodium, potassium, or chloride.

In a previously discussed trial, Cunnane et al. (1994), studying a number of nutritional endpoints, gave muffins providing either 0 or 50 g flaxseed/day to 10 healthy young adults (5 of each sex, mean age = 25 years) for 4 weeks in a randomized crossover design. Blood was sampled at baseline and at the end of each feeding period. During flaxseed consumption, α-linolenate, eicosapentaenoate, and docosapentaenoate were significantly increased in plasma phospholipids and triacylglycerols. There was no effect on antioxidant vitamins or lipid hydroperoxides in plasma. The absence of deleterious effects was noted.

Tarpila et al. (2002) completed a controlled double-blind crossover study to assess the effects of flaxseed on blood chemistry and serum lipids; the latter were discussed previously. Eighty food-company employees (18 males and 62 females, mean age 43 years) consumed diets that either did or did not contain 1.3% ground flaxseed (21.5 g flaxseed/day) and 5% flaxseed oil (along with 3-4% inulin and wheat fiber) for 4 weeks. Fifteen subjects continued an open trial with the flax-supplemented meals for 4 additional months. Blood parameters were measured at baseline, after both intervention periods, and at 2 and 4 months during the open study. The

measures included hemoglobin, leukocytes, aspartate and alanine amino transferases, alkaline phosphatase, creatinine, total and individual fatty acids, and enterolactone. No significant changes were seen in any of these measures except increased levels of enterolactone and altered concentrations of some of the individual fatty acids. Specifically, no differences were seen in myristic, pentadecanoic, palmitic, palmitoleic, stearic, linoleic, arachidonic, or docosahexaenoic acids; levels were lowered of oleic, vaccenic, and dihomo-γ-linoleic acid; and increased levels existed of α- and γ-linolenic acids and their metabolites eicosapentaenoic and docosapentaenoic acids. The authors concluded that the addition of flaxseed products to the diet may produce beneficial changes in blood levels of α-linolenic acid without causing deleterious effects.

Stuglin and Prasad (2005), discussed earlier, investigated the effects of short-term flaxseed on blood pressure, serum lipids, the hemopoietic system, and liver and kidney enzymes. Fifteen healthy males age 22-47 consumed 32.7 g flaxseed/day (in 3 muffins) for 4 weeks. Blood pressure and blood samples were collected before and after the feeding period. Concentrations of triacylglycerols increased and those of creatinine decreased, but most parameters remained unchanged: blood pressure, heart rate, hemoglobin, red blood cell counts, white blood cell counts, neutrophils; total, HDL, LDL, and VLDL cholesterol; bilirubin, aspartate aminotransferase, alkaline phosphatase, protein, albumin, glucose, and urea. The authors concluded that ingestion of 32.7 g flaxseed/day had no adverse effects on blood lipids, liver or kidney enzymes, or the hemopoietic system.

In a randomized double-blind placebo-controlled crossover study that was discussed in several sections above, Faintuch et al. (2007) administered 30 g/day of flaxseed flour or an equal mass of manioc flour (placebo) to 24 morbidly obese (average BMI = 47.1) outpatients. Each treatment period was 2 weeks with a 24-hour wash-out period. No intolerance was noted and there was no change in body weight. Neither treatment resulted in any change in biochemical indices: hemoglobin, white blood cell count, or serum albumin.

Fuchs et al. (2007) gave 7 healthy males age 20-40 400 mg/kg bw of ground flaxseed for 1 week. Fasting blood samples were taken 1 week before the start of the study, on days 0, 1, 3, and 7, and finally 2 weeks later. Plasma enterolactone was measured and protein was extracted from peripheral blood mononuclear cells including both monocytes and lymphocytes. Flaxseed significantly raised levels of peroxiredoxin and reduced levels of the long-chain fatty acid β-oxidation multienzyme complex and glycoprotein IIIa/II; these changes—which may be due to the lignan metabolite enterolactone—are indicators of reduced oxidative stress and reduced thrombotic potential and thus may indicate an anti-atherosclerotic effect. However, the effects of flaxseed began to decline for several participants between days 3 and 7, possibly indicating an adaptation in the microfloral population responsible for metabolizing the lignan SDG to enterolactone. Significant changes were seen in the levels of a total of 16 proteins; all were regarded as beneficial and none as adverse.

The above research is consistent in finding that consumption of flaxseed produces changes in serum lipids as discused in Section 4.3.1.2, particularly the distribution of fatty acids, without significant effects on other blood chemistry parameters.

4.3.2.6. Vascular Stress Response

Spence et al. (2003) used a random-sequence double-blind Latin square crossover study to compare the effects of 3 strains of flaxseed on responses to mental stress. Postmenopausal women age 54-70 years (n = 35) received 35 g/day of ground flaxseed. Each treatment period was 3 months, with a 1-month washout period between treatments. The 3 cultivars of flaxseed used were Linola 989 (high in SDG and low in ALA), Flanders (low in SDG and high in ALA), and AC Linora (intermediate in both SDG and ALA). The actual amounts of SDG and ALA provided daily by the 3 treatments is shown in Table 33. Compared to the baseline diet, all 3 flaxseed treatments significantly reduced blood pressure, peripheral resistance, plasma cortisol, and plasma fibrinogen during mental stress, but the Linola 989, high in SDG, was the most potent. The investigators suggested that flaxseed may be an alternative to hormone replacement therapy in reducing vascular risk among postmenopausal women. No adverse effects were reported in this study.

Table 33. Amounts of SDG and ALA Provided By Flaxseed Cultivars (Spence et al. 2003).

Cultivar	SDG (mg/day)	ALA (g/day)
Linola 989	450	0.3
Flanders	222	6.6
AC Linora	270	7.2
Source: Spence et al. (2003)		

4.3.2.7. Kidney Function

The Clark et al. (1995) study discussed above focused on the effect of flaxseed on a number of parameters in lupus nephritis patients. Eight patients (2 males and 6 females aged 32 to 72 years, mean = 44.5 years) consumed 15 g/day of milled flaxseed for 4 weeks, then 15 g twice a day (30 g/day) for the ensuing 4 weeks, and finally 15 g three times a day (45 g/day) for the next 4 weeks. Disease activity, blood pressure, plasma lipids, rheology, platelet activating factor (PAF)-induced platelet aggregation, renal function, and serum immunology were assessed. Disease activity was unchanged throughout the study. The 30-g/day dose of flaxseed was well tolerated and conferred benefits in renal function as assessed by serum creatinine, creatinine clearance, and urinary protein measures, as well as inflammatory and atherogenic mechanisms important in the pathogenesis of lupus nephritis.

In a randomized crossover trial, Clark et al. (2001) administered either 0 or 30 g/day of ground flaxseed to 23 patients with lupus nephritis for 1 year, followed by a 12-week washout period and the reverse treatment for 1 year. At baseline and 6-month intervals, serum creatinine; 12-hour urine albumin excretion and urine albumin to creatinine ratios; serum viscosity; and plasma lipids were measured. Compliance was assessed by counts of flaxseed sachets and serum phospholipids measures. Eight participants dropped out and only 9 of the 15 remaining subjects adhered to the flaxseed diet. Plasma lipids and serum viscosity were unaltered by the flaxseed supplementation whereas serum creatinine in the compliant patients during flaxseed

administration declined from a mean of 0.97±0.31 mg/dL to a mean of 0.94±0.30 mg/dL and rose in the control phase to a mean of 1.03±0.28 mg/dL (NS, p = 0.08). Despite the small and non-statistically significant effects, the authors concluded that flaxseed appears to be renoprotective in lupus nephritis. No adverse effects were reported.

In a study discussed previously, Stuglin and Prasad (2005) investigated the effects of short-term flaxseed on a number of endpoints, including liver and kidney enzymes. Fifteen healthy males age 22-47 consumed 32.7 g flaxseed/day (in 3 muffins) for 4 weeks. Blood samples were collected before and after the feeding period. Most parameters remained unchanged, including bilirubin, aspartate aminotransferase, alkaline phosphatase, albumin, and urea. The authors concluded that ingestion of 32.7 g flaxseed/day has no adverse effects and specifically no effects on liver or kidney enzymes.

The research discussed in this section confirms that flaxseed consumption has little effect on blood chemistry other than lipids, including liver and kidney enzymes. Its benefits for the management of patients with lupus nephritis are uncertain and appear to derive from its mild antiatherogenic and anti-inflammatory effects.

4.3.2.8. Cyanide Accumulation

In one of the few studies to investigate whether consumption of flaxseed results in any increase in serum levels of cyanide compounds or cadmium, Tarpila et al. (2002) completed a controlled double-blind crossover study to assess the effects of flaxseed consumption on serum thiocyanate and cadmium. Eighty food-company employees (62 women, 18 men, mean age 43 years) consumed diets that either did or did not contain 1.3% ground flaxseed and 5% flaxseed oil (along with 3-4% inulin and wheat fiber) for 4 weeks with a 4-week washout period. Fifteen subjects continued an open trial with the flax-supplemented meals for 4 additional months. The supplemented diets provided 21.5 g flaxseed/day. Blood parameters (hemoglobin, leukocytes, aspartate and alanine amino transferases, alkaline phosphatase, creatinine, serum lipids, fatty acids, and enterolactone) were measured at baseline, after both intervention periods, and at 2 and 4 months during the open study. The blood measures also included serum thiocyanate and cadmium. Non-cyanide-related findings of this study were discussed previously. No significant changes were seen in either measure. The authors concluded that the addition of flaxseed products to the diet may produce beneficial increases in serum enterolactone and n-3 fatty acids without causing deleterious effects.

Rosling (1993) reported that 2 male adults given 60 g/day ground flaxseed for 10 days exhibited a 7-fold increase in urinary excretion of thiocyanate, from an average of 32 μmol over the 3 days prior to dosing to an average of 247 μmol over the last 3 days of the study. No information was provided regarding preparation of the flaxseed.

Cunnane et al. (1993) conducted a series of 3 experiments to investigate the nutritional aspects of flaxseeds in the human diet. The nutritional aspects of these studies were discussed earlier, but one study also included a measure of thiocyanate excretion. In this experiment, muffins were prepared containing 25 g ground flaxseed or wheat flour and baked at 230°F for 15-18 minutes. The concentrations of malondialdehyde (MDA—a lipid peroxidation product)

and the cyanogenic glycosides linamarin, linustatin, and neolinustatin were measured both before and after baking. MDA levels were similar in both wheat and flaxseed muffins, and did not change after baking. Storage of the muffins for up to 2 years did not change the MDA levels. Analysis of the pre-baking flaxseed mucilage found 2-4 μg cyanogenic glycosides/g mucilage, but none could be detected after baking. Healthy female college students (5 per group) consumed either a supplement of flaxseed flour or the muffins baked with 25 g flaxseed flour, both treatments providing 50 g flaxseed/day. After 4 weeks the treatments were discontinued during a 2-week washout period. Analysis of thiocyanate excretion showed no significant effect of consumption of 50 g/day of flaxseed from either the supplement or the muffins.

In summary, while it appears that extremely high levels of consumption of flaxseed may increase the rate of excretion of thiocyanate, this effect was not seen in women who consumed 50 g flaxseed/day for 4 weeks. Additionally, the large body of animal and human research has not produced any indication of a cyanide-produced adverse effect.

4.3.3. Conclusions from Intervention Studies

As noted earlier, none of these studies was intended to evaluate the long-term safety of flaxseed or to determine human tolerance for large doses. Nevertheless, there is a large number of studies in which human participants consumed extremely high doses of flaxseed—as much as 50 g/day—with no observed adverse effects or signs of intolerance other than, in several studies, increased laxation (usually regarded as a beneficial effect of fiber-containing foods). It is also notable that large doses of flaxseed have not generally resulted in significantly increased levels of thiocyanate excretion.

A wide variety of beneficial effects has been suggested for flaxseed and human studies have been designed to assess such effects. The evidence regarding most effects must be regarded as suggestive rather than definitive. The evidence for effects usually regarded as fiber related—attenuation of the postprandial glycemic response, attenuation of blood cholesterol concentration, and laxation—is generally positive but weak. The evidence is sufficiently compelling, however, particularly regarding the laxation effect, that flaxseed may be appropriately regarded as a source of functional fiber.

Table 34. Human Studies

Citation	Study Design	Subjects	Dose	Duration	Results
Arjmandi et al. 1998	Double-blind placebo-controlled crossover: flaxseed v. sunflower seed	38 hyper-cholesterol-emic post-menopausal women	38 g/day flaxseed or sunflower seed	6 weeks for each treatment	Both treatments significantly reduced total cholesterol from baseline, but only the flaxseed significantly lowered LDL, as well as lipoprotein(a). Neither treatment affected HDL or triacylglycerol concentrations, and no adverse reactions were reported to either treatment.
Bierenbaum et al. (1993	Open label	15 hyper-lipidemic adults on vitamin-E therapy	15 g/day ground flaxseed	3 months	Total cholesterol and LDL were reduced; HDL was unchanged. Thrombin-stimulated platelet aggregation and lipid oxidation products were decreased with no adverse effects reported.
Brooks et al. 2004	Randomized parallel double-blind placebo-controlled: flaxseed v. soy flour	46 post-menopausal women	0 or 25 g/day ground flaxseed (50 mg/day SDG)	16 weeks	Urinary concentrations of 2-hydroxyestrone, but not of 16α-hydroxyestrone, increased significantly ($p < 0.05$) in the flaxseed group. No significant change in serum hormones or biochemical markers of bone metabolism was observed within or between the treatment groups. No adverse effects were reported.
Clark et al. 1995	Open label escalating dose	8 nephritis patients	15 g/day, 30 g/day, 45 g/day	4 weeks at each dose; 12 weeks total	3 subjects had difficulty with increased laxation at 45 g/day of flaxseed. Disease activity and blood pressure were unchanged. Total and LDL cholesterol and whole blood viscosity were reduced with 30 g and 45 g flaxseed/day. PAF-induced platelet aggregation was inhibited at all levels. Serum creatinine declined at 30 and 45 g, and creatinine clearance increased with increasing flaxseed dose with no change in serum urea. No changes were observed in total T helper (CD3+/CD4+), helper inducer (CD4+/CD29+), suppressor inducer (CD4+/CD45RA+) subsets, natural killer cells (CD3-/CD16+ and/or CD56+) or cytotoxic T cells (CD3+/CD16+ and/or CD56+). The 30 g/day dose was well tolerated and conferred benefits in renal function and in inflammatory and atherogenic mechanisms important in the pathogenesis of lupus nephritis. Other than the increased laxation at the 45-g/day level, no adverse effects were reported.

000127

Table 34. Human Studies

Citation	Study Design	Subjects	Dose	Duration	Results
Clark et al. 2001	Randomized crossover, no placebo: flaxseed v. nothing	23 lupus nephritis patients	30 g/day flaxseed	1 year	Only 9 subjects adhered to the flaxseed diet for the full year. Plasma lipids and serum viscosity were unaltered while serum creatinine declined in compliant patients. The authors concluded that flaxseed appears to be renoprotective in lupus nephritis. No adverse effects were reported.
Cunnane et al. 1993 (Study 1 of 3)	Randomized crossover, no placebo: flaxseed alone or in bread	9 healthy female college students	50 g/day flaxseed in supplement or in bread	4 weeks for each treatment; 8 weeks total	No difference in the effects of flaxseed as a supplement v. in bread; both increased ALA and total long-chain n-3 fatty acids in plasma and erythrocyte lipids. Both reduced total and LDL cholesterol. Thiocyanate excretion showed little effect of consumption of 50 g/day of flaxseed.
Cunnane et al. 1993 (Study 2 of 3)	Randomized crossover, no placebo: flaxseed oil capsules v. flaxseed flour	5 healthy females	flaxseed oil capsules or flour, both providing 20 g/day oil	4 weeks for each treatment; 8 weeks total	Changes in plasma ALA were equivalent, suggesting that the ALA in ground flaxseed is highly available.
Cunnane et al. 1993 (Study 3 of 3)	Open label, glucose challenge	6 healthy men and women	50 g flaxseed followed by 25 g extracted flaxseed mucilage	Single meal with flaxseed, single meal with extracted flaxseed mucilage	The incremental area under the blood-glucose curve was 28% lower after consumption of flaxseed bread than after wheat bread. The addition of flaxseed mucilage reduced the area under the blood-glucose curve by 27%.

000128

Table 34. Human Studies

Citation	Study Design	Subjects	Dose	Duration	Results
Cunnane et al. 1994	Randomized crossover, no placebo: flaxseed v. nothing; with glucose challenge	10 healthy young adults (5 M, 5 F)	50 g/day flaxseed	4 weeks for each period for men; 1 menstrual period for women	Compliance was good. Flaxseed muffins did not differ from controls in thiobarbituric acid-reactive substances. During flaxseed consumption, α-linolenate, eicosapentaenoate, and docosapentaenoate increased in plasma phospholipids and triacylglycerols; α-linolenate also increased in adipose tissue. LDL cholesterol decreased and urinary lignan excretion increased more than 5-fold. No effect on antioxidant vitamins or lipid hydroperoxides in plasma. The flaxseed was mildly laxative, with bowel movements increasing 30% in frequency. The absence of deleterious effects was noted.
Dahl et al. 2005 (Study 1 of 2)	Randomized parallel double-blind placebo-controlled: flaxseed v. psyllium	26 healthy adults age 18-60 years	5 g/day flaxseed for 3 days, then increasing to 15 g/day	2 weeks tota	Both fiber treatments (flaxseed and psyllium) significantly increased fecal mass over baseline; there was no difference between treatments.
Dahl et al. 2005 (Study 2 of 2)	Randomized parallel double-blind placebo-controlled single meal with glucose challenge	11 healthy adults age 20-26 years	Bread with 50 g available carbohydrate with or without flaxseed	Single meal	The flax meal treatment resulted in a reduced area under the curve of glycemic response as well as a lower peak response. Together, these 2 experiments demonstrate that the soluble and insoluble fiber present in flax meal produces the functional effects to be expected.
Demark-Wahnefried et al. 2001	Pilot open label	25 prostate-cancer patients awaiting prostatec-tomy	30 g/day flaxseed	21 to 77 days until surgery (average = 34 days)	Total cholesterol was significantly lowered, as were total testosterone and free androgen index. Levels of PSA were unaffected. As compared with historical controls, both proliferation rate and apoptosis were favorably affected in a time-in-treatment-dependent manner.

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Table 34. Human Studies

Citation	Study Design	Subjects	Dose	Duration	Results
Dodin et al. 2005	Randomized parallel double-blind placebo-controlled: flaxseed v. wheat germ	101 healthy menopausal women aged 45-65 years; 99 women consumed placebo	40 g/day flaxseed	12 months	10% of women in the flaxseed group and 5% in the wheat-germ group dropped out due to digestive problems. Total and HDL cholesterol were reduced in the flaxseed group; there was no change in triacylglycerols. Flaxseed did not suppress progressive bone loss, but reduced menopausal symptoms. Aside from the digestive issues likely due to the high level of dietary fiber provided by 40 g flaxseed/day, no adverse side effects were observed.
Faintuch et al. 2007	Randomized double-blind [lacebo-controlled crossover: flaxseed v. manioc flour	24 obese (average BMI = 47.1) outpatients	30 g/day flaxseed	2 weeks for each treatment	No intolerance was noted. No change in biochemical or hormonal indices: hemoglobin, white blood cell count, total cholesterol, HDL, LDL, VLDL, triacylglycerols, glucose, serum albumin, insulin, or leptin. Flaxseed reduced the inflammatory markers C-reactive protein, serum amyloid A, and fibronectin, but not complement fraction C3, or complement fraction C4. There were no adverse effects on biochemical and hormonal endpoints.
Frische et al. 2003	Randomized crossover: 4 treatments—nothing, flaxseed, wheat brand, flaxseed + wheat bran	16 healthy premeno-pausal women aged 20-38 years	10 g/day flaxseed; 10 g/day flaxseed + 28 g/day wheat bran	2 full menstrual cycles for each arm; 4 cycles total with flaxseed	Lignan excretion increased during periods of flaxseed consumption, but there were no changes from any treatment on serum hormones (17β-estradiol, dehydroepiandrosterone sulfate, estrone, and progesterone) or sex hormone binding globulin.

000130

Table 34. Human Studies

Citation	Study Design	Subjects	Dose	Duration	Results
Fuchs et al. 2007	Open label proteomic study.	7 healthy males age 20-40	400 mg/kg ground flaxseed (~30 g/day)	1 week	Flaxseed significantly raised levels of peroxiredoxin and reduced levels of the long-chain fatty acid β-oxidation multienzyme complex and glycoprotein IIIa/II. No adverse effects were seen in the proteome.
Griel et al. 2007	Randomized, double-blind, balanced order, 3-period crossover: "average" diet, high-ALA diet with flaxseed, high-linoleic acid diet	23 over-weight and hyper-cholesterol-emic male and female adults	20 g/day flaxseed	6 weeks for each treatment	The flaxseed (+ walnuts + walnut oil) diet reduced N-teleopeptides compared with the "average American" diet while the linoleic-acid diet was intermediate. There was no effect on bone-specific alkaline phosphatase levels, indicating that rates of bone formation were unchanged, but bone resorption was decreased.
Haggans et al. 1999	Randomized crossover, no placebo: 2 flaxseed doses v. nothing	28 post-menopausal women	5 or 10 g/day ground flaxseed	7 weeks at each dose; 14 weeks total	Urinary excretion of the estrogen metabolite 2-hydroxyestrogen increased, while that of 16α-hydroxyestrone did not. The authors concluded that flaxseed may have chemoprotective effects in postmenopausal women; no adverse effects were reported.
Haggans et al. 2000	Randomized crossover: 4 treatments—nothing, flaxseed, wheat brand, flaxseed + wheat bran	16 healthy premeno-pausal women aged 20-38 years	10 g/day flaxseed; 10 g/day flaxseed + 28 g/day wheat bran	2 full menstrual cycles for each arm; 4 cycles total with flaxseed	Flaxseed, but not wheat bran, increased excretion of 2-hydroxyestrogen and the ratio of the two metabolites (2-hydroxyestrogen/16α-hydroxyestrone), regarded as chemoprotective against breast cancer risk.
Hallund et al. 2006	Randomized, double-blind, placebo-controlled crossover: muffins with SDG or placebo	22 post-menopausal women	500 mg/day SDG extracted from flaxseed	6 weeks for each period	The SDG intervention increased both plasma and urine levels of enterolactone; no other measures (total cholesterol, LDL, HDL, triacylglycerols, antioxidant capacity, ferric-reducing ability, and lipoprotein oxidation lag time) were significantly affected. No adverse effects attributable to the intervention were reported.

000131

Table 34. Human Studies

Citation	Study Design	Subjects	Dose	Duration	Results
Hutchins et al. 2000	Randomized crossover, no placebo: 2 doses of flaxseed or nothing	31 healthy post-menopausal women aged 52-82 years	5 or 10 g/day flaxseed	7 weeks at each dose; 14 weeks total	Excretion of matairesinol was not affected by flaxseed consumption, but excretion of enterolactone and enterodiol were increased in a dose-dependent manner.
Jenkins et al. 1999	Randomized placebo-controlled crossover: flaxseed v. wheat bran	29 hyper-lipidemic subjects (22 males and 7 post-menopausal women)	50 g/day partially defatted flaxseed	3 weeks for each treatment	Reductions were seen in total and LDL cholesterol and apolipoprotein B along with an increase in triacylglycerols. Thiol concentrations were lowered. No changes were seen in blood pressure, apolipoprotein A-1, protein carbonyl content, or agonistic or antagonistic activities of serum androgen and progestin. These effects were attributed to the mucilage content of the flaxseed.
Kelley et al. 1991	Randomized crossover, no placebo: flaxseed v. nothing	10 healthy males age 21-37	31.7 g/day flaxseed oil	56 days for each treatment	Flaxseed oil suppressed proliferation of peripheral blood mononuclear cells cultured with T-cell mitogen, but concentrations of granulocytes, lymphocytes, and monocytes did not differ, nor was there any change in numbers of circulating T cells (Leu-4, CD3), helper-inducer cells (Leu 3a, CD4), suppressor/cytotoxic cells (Leu-2a, CD8), or B cells (Leu-12, CD19). No differences were seen in concentrations of IgG. Flaxseed oil suppressed some indices of cell-mediated immune function without affecting markers of humoral immunity. No signs of health risks were observed.
Kurzer et al. 1995	Randomized crossover, no placebo: flaxseed v. nothing	13 pre-menopausal women (average age = 28)	10 g/day flaxseed	3 full menstrual cycles	Flax consumption increased fecal excretion of the lignans enterodiol, enterolactone, and matairesinol, but there was no effect on fecal excretion of the isoflavonoids daidzein, equol, genistein, and O-demethylangolensin.

000132

Table 34. Human Studies

Citation	Study Design	Subjects	Dose	Duration	Results
Lampe et al. 1994	Randomized crossover, no placebo: flaxseed v. nothing	18 pre-menopausal women age 20-34	10 g/day flaxseed	3 full menstrual cycles	Flaxseed resulted in increased urinary excretion of enterolactone, enterodiol, and total lignan, but no change in excretion of matairesinol or the isoflavonoids tested—daidzein, equol, genistein, and *O*-demethylangolensin.
Lemay et al. 2002	Randomized crossover: 2 treatments; flaxseed v. hormone therapy	25 hyper-cholesterol-emic menopausal women	40 g/day flaxseed	2 months for each treatment	No difference in the two regimens regarding decreasing menopausal symptoms, reducing glucose and insulin levels, or reducing LDL cholesterol, but only the hormone-replacement therapy was effective in increasing HDL cholesterol and apolipoprotein A-1, as well as sex hormone binding globulin, and in lowering plasminogen activator inhibitor type 1.
Lewis et al. 2006	Randomized, double-blind, placebo-controlled	87 women 1-8 years post-menopause	25 g/day flaxseed providing 50 mg lignan	16 weeks	Hot flashes were significantly less severe with flaxseed than with the control muffins, but there was no significant effect on the quality-of-life instrument.
Lucas et al. 2003	Randomized, parallel, double-blind, placebo-controlled: flaxseed v. wheat-flour	36 post-menopausal women	40 g/day flaxseed	3 months	GI problems were more frequent in the wheat-flour group while palatability issues were more frequent for flaxseed. Flaxseed lowered both total and non-high-density cholesterol and apolipoproteins A-1 and B; other measures were not significantly affected. Flaxseed had no effect on bone metabolism, nor did it exert any estrogenic properties as assessed by the lack of effects on circulating levels of sex hormones.

0001133

Table 34. Human Studies

Citation	Study Design	Subjects	Dose	Duration	Results
Nesbitt et al. (1999)	Randomized crossover: doses of flaxseed, raw v. processed	9 healthy pre-menopausal women age 20-40	5, 15, or 25 g/day raw ground flaxseed or 25 g/day processed flaxseed	7 days for each arm	Dose-dependent plasma levels and urinary excretion of lignan (primarily enterodiol) were observed, with plasma levels peaking approximately 9 hours after ingestion. There were no difference in lignan concentrations between ingestion of raw and processed flaxseed.
Phipps et al. (1993)	Randomized crossover: no placebo: flaxseed v. nothing	18 healthy women age 20-34 years	10 g/day flaxseed	3 full menstrual cycles for each arm	The flax cycles were consistently associated with longer luteal phase of the menstrual cycle, but there were no changes in excretion of enterolactone or enterodiol or in blood levels of estradiol, estrone, testosterone, progesterone, dehydroepiandrosterone sulfate, or sex hormone-binding globulin. Flaxseed ingestion resulted in a decreased tendency to ovarian dysfunction.
Rosling 1993	Open label	2 male adults	60 g/day ground flaxseed	10 days	Exhibited a 7-fold increase in urinary excretion of thiocyanate from an average of 32 µmol to 247 µmol.
Shultz et al. (1991)	Open label	6 healthy males age 25-42	13.5 g/day flaxseed flour	6 weeks	Urinalysis found enterodiol and enterolactone raised by a factor of 7-28-fold over baseline, with enterolactone 5-10-fold greater than enterodiol. Blood showed no change in total testosterone, free testosterone, or sex-hormone-binding globulin and no effects on liver function, plasma glucose, blood urea nitrogen, creatinine, alkaline phosphatase, calcium, phosphorus, sodium, potassium, chloride, triacylglycerols, or total cholesterol.

Table 34. Human Studies

Citation	Study Design	Subjects	Dose	Duration	Results
Snyder et al. 2007	Randomized placebo-controlled	161 prostate cancer patients with prostatectomy surgery scheduled for at least 3 weeks later	30 g/day flaxseed	Average of 30-31 days	The men receiving flaxseed had higher intakes of ALA and higher n-3/n-6 ratios than the other groups, and had higher levels of EPA in erythrocytes and prostatic tissue as well as higher n-3/n-6 ratios in prostatic tissue. The authors concluded that conversion of ALA to EPA occurs with flaxseed and may support its use in the treatment of prostate cancer.
Spence et al. (2003)	Random-sequence double-blind Latin square crossover: 3 flaxseed strains (Linola 989, AC Linora, Flanders)	35 post-menopausal women age 54-70 years	35 g/day flaxseed	3 months for each cultivar; 9 months total	All 3 flaxseed treatments reduced blood pressure, peripheral resistance, plasma cortisol, and plasma fibrinogen during mental stress, but the strain high in SDG was the most potent. Concluded that flaxseed may be an alternative to hormone replacement therapy in reducing vascular risk among postmenopausal women. No adverse effects were reported in this study.
Stuglin and Prasad (2005)	Open-label study	15 healthy males age 22-47	32.7 g/day flaxeed	4 weeks	Blood concentrations of triacylglycerols increased and those of creatinine decreased, but most parameters remained unchanged: blood pressure, heart rate, hemoglobin, red blood cell counts, white blood cell counts, neutrophils; total, HDL, LDL, and VLDL cholesterol; bilirubin, aspartate aminotransferase, alkaline phosphatase, protein, albumin, glucose, and urea. Ingestion of 32.7 g flaxseed/day had no adverse effects on blood lipids, liver or kidney enzymes, or the hemopoietic system.

Table 34. Human Studies

Citation	Study Design	Subjects	Dose	Duration	Results
Tarpila et al. (2002)	Controlled double-blind crossover; no placebo: flaxseed v. nothing	80 food-company employees (62 women, 18 men, mean age 43 years); 15 in follow-up	1.3% ground flaxseed and 5% flaxseed oil	4 weeks each arm followed by 4-month open label follow-up	No changes were seen in blood levels of hemoglobin, leukocytes, aspartate or alanine amino transferases, alkaline phosphatase, creatinine, total cholesterol, HDL or LDL cholesterol, triacylglycerols, total fatty acids, or in thiocyanate or cadmium. There were increased levels of enterolactone. No differences were seen in myristic, pentadecanoic, palmitic, palmitoleic, stearic, linoleic, arachidonic, or docosahexaenoic acids; levels were lowered of oleic, vaccenic, and dihomo-γ-linoleic acid; and increased levels existed of α- and γ-linolenic acids and their metabolites eicosapentaenoic and docosapenaenoic acids. The authors concluded that the addition of flaxseed products to the diet may produce beneficial changes in blood levels of enterolactone and α-linolenic acid without causing deleterious effects.
Thompson et al. (2005)	Randomized, parallel, double-blind, placebo-controlled: flaxseed v. whole wheat flour	19 women (average age 70 years) with newly diagnosed breast tumors	25 g/day flaxseed	Until surgery, an average of 32 days	The flaxseed group had higher lignan excretion, and exhibited significant reductions in tumor cell proliferation and c-erbB2 expression and an increased level of apoptosis. Concluded that flaxseed has the potential to reduce breast-cancer tumor growth and invasiveness and is a safe and tolerable intervention.

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4.4. Observational Studies

A number of observational studies—prospective and retrospective cohort studies as well as nested case-control studies—have addressed the question of a possible relationship between ALA intake and the risk of prostate cancer, and one study examined associations between consumption of phytoestrogens such as the lignans found in flaxseed and prostate cancer risk.

Giovannucci et al. (1993) studied the association between fat intake and prostate cancer using data from the Health Professionals Follow-up Study, a prospective cohort of 51,529 U.S. men age 40-75 years who completed a "validated" food-frequency questionnaire in 1986. (It should be noted that the validity of food-frequency questionnaires is seriously questioned, particularly for estimates that may depend on levels of consumption of specific foods as opposed to broad food categories. See, for example, Schaefer et al. 2000). As of 1990, 300 cases of prostate cancer were identified in the cohort. Associations were found between advanced prostate cancer and consumption of animal fat but not vegetable fat. Similar associations were also found with saturated fat, monounsaturated fat, and ALA, but not linoleic acid.

In 1982, at the start of the Physicians' Health Study, 14,916 U.S. male physicians provided plasma samples. In a nested case-control design, Gann et al. (1994) compared plasma fatty acids of 120 men who later developed prostate cancer with 120 matched controls. There was an overall trend for men with higher levels of plasma ALA to be in the prostate cancer group rather than the control group, although the relative risk of the highest quartile was lower than that of the 2nd or 3rd quartiles. The authors concluded that the effects of ALA warrant further study.

A population-based case-control study was conducted in Orebro County, Sweden from 1989 to 1994, in which 526 patients with newly diagnosed prostate cancer were compared with 526 randomly selected matched controls (Andersson et al. 1996). While positive associations with prostate cancer were found for total energy intake, total fat, protein, retinol, and zinc, the association with ALA was insignificantly negative.

Harvei et al. (1997) used a nested case-control study to study relationships between blood lipids and later development of prostate cancer. Among blood donors to the Janus serum data bank in Norway, 141 later developed prostate cancer; they were matched with other donors who did not develop cancer. Positive associations were found for palmitoleic acid, palmitic acid, and ALA. No information was available regarding diet or the sources of these fatty acids, all of which are found in foods of both animal and plant origin.

The Netherlands Cohort Study, including 58,279 males age 55-69 years, was initiated in 1986, at which time participants completed a 150-item semiquantitative food-frequency questionnaire. After 6.3 years of follow-up, Schuurman et al. (1999) identified 642 incident prostate carcinoma cases for comparison with 1525 matched controls in a case-cohort study. No individual fatty acids had statistically significant associations with prostate cancer, but oleic acid showed a positive association and ALA a negative one. Indeed, one of the analyses of ALA showed a statistically significant reduced risk of prostate cancer with increasing intake, but this

reduction fell to non-significance when the data were adjusted for socioeconomic status, family history of prostate cancer, energy intake, and energy-adjusted fat intake.

A case-control study was conducted in Barcelona, Spain, in which 217 incident cases of prostate cancer were matched with 434 controls by age and residence (Ramon et al. 2000). A semiquantitative food-frequency questionnaire was used to assess food intake. A significant positive association was found between prostate cancer risk and consumption of animal fat and ALA, and a negative association was found with vitamin C. Sources of ALA were not identified, but the positive association with animal fat and negative association with vitamin C may point to animal rather than plant sources.

De Stefani et al. (2000) conducted a case-control study on diet and prostate cancer in Uruguay, in which 217 advanced prostate cancer cases were matched with 431 controls on age, residence, and urban/rural status. All participants were administered a 64-item food-frequency questionnaire. Positive associations were found for total fat intake, saturated and monounsaturated fats, and ALA; a negative association was found for oleic acid. In this study, as is evident from the associations, meat rather than vegetable-based foods was the primary source of ALA, although the authors concluded that the association with prostate cancer was significant for ALA from both sources.

In a small case-control study with 67 prostate cancer patients matched with 156 population-based controls, Newcomer et al. (2001) compared levels of erythrocyte membrane fatty acids. Positive associations were found with higher levels of ALA, linoleic acid, and total n-6 fatty acids. As was noted by Godley et al. (1996), the n-3 fatty acid composition of erythrocyte membranes appear to be generally valid indicators of n-3 fatty acid intakes, but it is not yet proven that they are valid measures of intake of the individual fatty acids.

Mannisto et al. (2003) investigated the effects of fatty acids on the risk of prostate cancer in a nested case-control study. In a cohort of 29,133 male smokers age 50-69 years (from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study), 246 men with diagnosed prostate cancer were identified. One control was selected and matched by age for each of these cases; of these pairs, 198 had baseline serum samples available for both members. Dietary intakes of fatty acids were assessed from a self-administered dietary questionnaire, and fatty acids of serum cholesterol esters were measured using capillary gas chromatography. No association was found between either dietary intake or serum levels of ALA and the risk of prostate cancer.

From a cohort of 47,866 males age 40-75 years with no cancer at baseline (from the Health Professionals Follow-Up Study) followed from 1986 to 2000, Leitzmann et al. (2004) identified 2965 new cases of prostate cancer, 448 of which were advanced. ALA intake, as assessed by 3 administrations of a semiquantitative food-frequency questionnaire, was not related to the risk of total prostate cancer; however, extreme levels of ALA intake from beef, pork, lamb, cheese, skim milk, mayonnaise, salad dressings, and margarine were positively related to the risk of advanced prostate cancer. It should be noted that none of these food sources include as constituents either flaxseeds or flaxseed oil.

Associations among dietary phytoestrogens, serum enterolactone, and the risk of prostate cancer were examined in the Cancer Prostate Sweden Study, a case-control study (Hedelin et al. 2006). Dietary intake questionnaire data were available for 1,499 prostate cancer cases and 1,130 controls, with serum enterolactone levels in a sub-group of 209 cases and 214 controls. Logistic regression was used to estimate multivariate odds ratios for associations with risk of prostate cancer. High intake of food items rich in phytoestrogens (flaxseed, sunflower seeds, berries, peanuts, beans and soy) was associated with a decreased risk of prostate cancer. The odds ratio comparing the highest to the lowest quartile of intake was 0.74 ($p=0.01$). Intermediate serum levels of enterolactone were associated with a decreased risk of prostate cancer. The authors concluded that, "Our results support the hypothesis that certain foods high in phytoestrogens are associated with a lower risk of prostate cancer." In this study, the highest contributors to isoflavonoids were soy products while the primary contributors to lignan intakes were flaxseed and rye bread.

The significance of this body of research is discussed in the following section.

4.5. Potential for Adverse Effects from Overconsumption of α-Linolenic Acid

The IOM's Panel on Macronutrients (IOM 2002) did not set a Tolerable Upper Intake Level (UL) for α-linolenic acid (ALA) or for total n-3 fatty acids because quantitative data were lacking. However, FDA has previously determined that a daily intake of up to 3 g/day of EPA and DHA combined is without significant likelihood of harm (FDA 1997b).

A large number of studies have addressed the question of the bioconversion of ALA (18:3 n-3) to EPA and DHA. The initial conversion of ALA to EPA begins with the introduction of a double bond by the action of Δ6-desaturase (IOM 2002), producing 18:4 n-3; this is the rate-limiting reaction of the pathway (Williams and Burdge 2006). The conversion continues with the addition of C_2 by elongase activity, followed by a second desaturation by Δ5-desaturase to form EPA. The formation of DHA requires a number of additional steps (IOM 2002) and is biologically difficult. A review of these studies by Williams and Burdge (2006) led them to conclude that the best estimate of the maximum conversion of ALA to EPA in adults is approximately 8% (and often less). Further transformation to DHA is less than 1% efficient in males but perhaps as high as 9% in females..

Accepting the maximum figure of 8% for the bioconversion of ALA to EPA, about 37 g of ALA is needed to produce 3 g of EPA. Since ALA constitutes about 25% of whole or ground flaxseed, ingestion of about 150 g of flaxseed would be needed to obtain sufficient ALA to be converted to 3 g of EPA. This is far higher than the likely consumption of flaxseed resulting from its intended use.

A number of cohort studies have found correlational relationships that allegedly implicate high intakes of ALA in increased risk of prostate cancer. A review of this literature (Brouwer et al 2004) noted that no clinical trials report any relationship of ALA intake with prostate cancer (or with prostate-specific antigen), but concluded that the apparent association seen in cohort

studies is "of concern and warrants further study." They conceded, however, that, "It is quite uncertain at present whether the effect on prostate cancer is real" (Brouwer et al. 2004, p 921).

This review also investigated associations between ALA intake and fatal coronary heart disease (CHD). For the CHD review, the authors limited their investigation to prospective cohort studies, because "these are regarded as having the strongest design of all observational studies, and also because case-control studies do not provide reliable information on diet and fatal heart disease" (Brouwer et al. 2004, p 919). Nevertheless, when the authors turned to the meta-analysis of ALA and the risk of prostate cancer, they selected *all* observational studies, prospective or retrospective, as well as case-control studies.

Their summary of Mannisto et al. (2003) was curious. Brouwer et al. (2004) cited the findings of this study as "the relative risk for prostate cancer in the 4th versus the 1st quartile of ALA intake was 1.16" (p 921). Mannisto et al. (2003), on the other hand, reported their own findings as, "No differences were found for mean intakes of fatty acids between the cases and the controls" (p 1423) and "No significant associations were found between serum ALA or any other fatty acid and prostate cancer risk" (p 1424).

The Brouwer et al. (2004) analysis included 9 observational studies that investigated prostate cancer incidence or prevalence and intake of ALA or blood levels of ALA, all of which were discussed above. It is noteworthy that 2 of the largest studies, Schuurman et al. (1999) with 642 prostate cancer cases and Andersson et al. (1996) with 526 patients, found negative associations between ALA and prostate cancer. Further, all of these studies have been observational rather than controlled, and most of them have been retrospective or case-control rather than prospective. While there may be some cause for concern with extremely high levels of intake of ALA, it clear that a *causative* role of this fatty acid has not been shown, particularly at the levels of intake anticipated to result from the intended use of whole and ground flaxseed. In must also be noted that a similar case-control study of associations between consumption of foods rich in lignans and isoflavonoids, discussed above, found that men consuming greater amounts of these foods had a lower risk of prostate cancer (Hedelin et al. 2006).

There have been 2 prospective intervention studies (both discussed earlier) regarding flaxseed ingestion and prostate cancer, and both have shown small but beneficial rather than adverse effects. In Demark-Wahnefried et al. (2001), 25 prostate-cancer patients awaiting prostatectomy were placed on a low-fat (20% of energy or less) diet supplemented with 30 g/day ground flaxseed for the 21 to 77 days (average = 34 days) before surgery; only historical controls were available. At baseline and follow-up levels of prostate-specific antigen (PSA), testosterone, free androgen index, and total serum cholesterol were measured, and tumor proliferation and apoptosis were compared with historical data. Total cholesterol was significantly lowered, as were total testosterone and free androgen index. Levels of PSA were unaffected. As compared with historical controls, both proliferation rate and apoptosis were favorably affected by the flaxseed intervention.

In a similar study reported in 2007 (Snyder et al. 2007), the effects of flaxseed supplementation and dietary fat restriction on the progression of prostate cancer were examined in 161 prostate cancer patients. Patients received 30 g flaxseed/day, dietary fat restriction to

<20% of total energy, flaxseed supplementation + dietary fat restriction, or control. Erythrocytes and prostatic tissue were analyzed for fatty acids. The men receiving flaxseed had higher levels of EPA in erythrocytes and prostatic tissue as well as higher n-3/n-6 ratios in prostatic tissue. The authors concluded that conversion of ALA to EPA occurs with flaxseed and may support its use in the treatment of prostate cancer.

ALA is regarded as an essential fatty acid. As was discussed above, the IOM Panel on Macronutrients (IOM 2002) established an Adequate Intake for ALA at 1.6 g/day for adult males and 1.1 g/day for adult females. Also as noted previously, Gebauer et al. (2006) cited 5 other authoritative bodies that have promulgated dietary recommendations regarding ALA, all of them higher than the AI set by the IOM, which is simply the average consumption in the U.S. population where there is no obvious sign of ALA deficiency. The recommendations summarized by Gebauer et al. (2006) were:

- 3 g ALA/day by a 1989 North Atlantic Treaty Organization workshop;
- 2.2 g ALA/day by a workshop of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) in 1999;
- 2 g ALA/day by the Eurodiet Commission in 2000;
- 1.8 g ALA/day by the French Apports Nutritionnels Conseilles in 2001; and
- 1.6 g ALA/day by ISSFAL in 2004.

4.6. Potential for Adverse Effects from Overconsumption of Total Fiber

The Panel on Macronutrients (IOM 2002) reviewed the published literature regarding the potential for adverse effects due to overconsumption of dietary fiber and due to overconsumption of functional fiber. One area of particular emphasis was the effect of fiber intake on mineral bioavailability, particularly calcium, magnesium, iron, and zinc. The panel concluded that there is little evidence that fiber itself, absent phytate, has adverse effects on mineral absorption or status. The panel also concluded that intake of dietary fiber at levels in excess of 40 g/day do not result in significant increases in gastrointestinal distress absent special circumstances such as pancreatic disease. The panel summarized its discussion of dietary fiber as follows:

> "*Dietary Fiber* can have variable compositions and therefore it is difficult to link a specific fiber with a particular adverse effect, especially when phytate is also often present. It is concluded that as part of an overall healthy diet, a high intake of *Dietary Fiber* will not produce significant deleterious effects in healthy individuals. Therefore, a Tolerable Upper Intake Level is not set for *Dietary Fiber*" (IOM 2002, p 7-43).

The IOM (2002) panel also examined the need to set a Tolerable Upper Intake Level (UL) for isolated and synthetic fibers (functional fiber), because it is possible to concentrate

large amounts of these fibers in foods, beverages, and supplements. The panel suggested that it would be informative to develop projections regarding the potential contribution of functional fiber to daily total fiber intake. Noting that functional fiber, like dietary fiber, is not digested by mammalian enzymes and passes into the colon, the panel determined that any potentially deleterious effects of functional fiber ingestion would be on the interaction with other nutrients in the gastrointestinal tract. The panel summarized its review as follows:

> "While occasional adverse gastrointestinal symptoms are observed when consuming one of the above isolated or synthetic fibers, serious chronic adverse effects have not been observed. Furthermore, due to the bulky nature of fibers, excess consumption is likely to be self-limiting. Therefore, a UL was not set for these individual fibers" (IOM 2002, p 7-46).

4.7. Potential for Adverse Effects from Exposure to Cyanide from Flaxseed

As was discussed earlier, flaxseed—like many other edible plants (White et al. (1998) estimated as many as 12,000 plant species)—contains cyanogenic glycosides. If these glycosides are not removed (by washing) or denatured (by heat), they can produce cyanide in the gastrointestinal tract as a result of enzymatic hydrolysis by β-glucosidases produced by gut microbiota. Bhatty (1993) found a range of 0.06—0.12 ppm (mean 0.09 ppm) HCN in a variety of flaxseed cultivars grown at different locations during different years.

Montgomery (1980) reported the cyanide yield of a variety of different foods. Similarly, in a background document for evaluation of cyanide in drinking water, the World Health Organization discussed existing exposures to cyanide from a number of sources, including foods (WHO 2007). The cyanide concentrations in food products were summarized in a table, a portion of which is reproduced below as Table 35, along with data from Montgomery (1980).

Table 35. Cyanide Concentrations in Food Products.

Type of Food Product	Cyanide Concentration (ppm)
Cereal grains and products	0.001—0.45[1]
Soy protein products	0.07—0.3[1]
Homemade cherry juice from pitted fruits	5.1[1]
Commercial cherry juice	4.6[1]
Commercial apricot juice	2.2[1]
Commercial prune juice	1.9[1]
Cassava (bitter) whole tubers	380[1]
Cassava (sweet) whole tubers	445[1]
Sorghum (whole immature plant)	2400[1]; 2500[2]
Bitter almonds	2500[2]
Bamboo (immature shoot tip)	7700[1]; 8000[2]
Lima beans (white American)	100[2]
Lima beans (from Java, Burma, or Puerto Rico)	2000—3000[1]

1. Source: WHO (2007)
2. Source: Montgomery (1980)

The average concentration of cyanide in flaxseed, 0.09 ppm, is well within the range commonly found in plants used as human food. In its 1992 review, JECFA (1993) determined that data were lacking to establish a safe level of intake of cyanogenic glycosides. However, the Committee concluded that a level of up to 10 ppm hydrogen cyanide in the Codex Standard for cassava flour is not associated with acute toxicity. This level is more than 100 times that found in flaxseeds. Additionally, as was discussed previously, while one study found a statistically significant (but non-toxic) rise in urinary excretion of thiocyanate after 10 days' consumption of 60 g/day of flaxseed, two other studies found no change in thiocyanate excretion after 4 weeks ingestion of either 50 g/day of flaxseed or flaxseed constituting 1.3% of the diet.

4.8. Potential Interactions of Flaxseed with Drugs

Like other foods, flaxseed has the potential to interact with drugs, particularly those taken orally. The Natural Standard Research Collaboration (Basch et al. 2007) suggested that flaxseed, like other sources of dietary fiber, may decrease the absorption of coadministered oral medications. Although no human studies have addressed the issue, the ALA found in flaxseed, like other n-3 fatty acids, has the potential to reduce platelet aggregation, increase bleeding time, and reduce inflammation, and thus may potentiate the effect of anticoagulants, antiplatelet

agents, and nonsteroidal anti-inflammatory drugs. However, as noted above, the conversion of ALA to EPA and DHA is inefficient and it is likely that such effects would be minor.

Basch et al. (2007) also suggested the possibility that flaxseed would potentiate the effect of antihyperlipidemic or antihypertensive agents, although the authors admitted that (as discussed above) the lipid-lowering and blood-pressure lowering effects of flaxseed are questionable. A higher degree of likelihood exists that flaxseed, which does affect laxation, may act additively with laxatives or stool softeners.

A Health Canada website (2007) lists common foods that may interact with warfarin. Flaxseed is included in this list along with cranberry juice, onions, garlic, and products containing soy protein.

Because flaxseed is an excellent source of lignans which possess *in vitro* antioxidant and possible estrogen receptor agonist/antagonist properties, Basch et al. (2007) suggested the possibility (not supported by direct evidence) that flaxseed ingestion might affect the function of oral contraceptives, hormone replacement therapy, or other estrogen-related agents.

5. SAFETY ASSESSMENT AND GRAS DETERMINATION

5.1. Introduction

This chapter presents an assessment that demonstrates that the intended uses of whole and milled flaxseed are safe, and are GRAS, for direct addition to conventional foods at levels limited only by cGMP to increase the dietary intake of α-linolenic acid (ALA) and total fiber. Although there is persuasive evidence of the safety of the intended use of milled or whole flaxseed as food ingredients from the history of consumption of foods containing flaxseed, this GRAS determination is based on scientific procedures. Under this approach, the history of consumption of flaxseed is regarded as one source of supporting information, along with the findings of *in vitro*, animal, and human studies addressing the safety of flaxseed ingestion.

This safety assessment and GRAS determination entail two steps. In the first step, the safety of flaxseed under its intended conditions of use is demonstrated. Safety is established by demonstrating that the likely intake of flaxseed under its intended conditions of use is within levels of intake that have been shown to be safe. In the second step, whole and ground flaxseed are determined to be GRAS by demonstrating that the safety of these products under their intended conditions of use is generally recognized among qualified scientific experts and is based on publicly available and accepted information.

5.2. Previous FDA Opinions on the Safety of Flaxseed Products

On February 7, 1983, FDA responded to an inquiry from Harvest Foods Ltd. of Saskatoon, Saskatchewan, Canada, regarding the status of flaxseed for food use in the U.S. (FDA 1983). In its response, FDA noted that, "Untreated (non-defatted) flaxseed has been used for food in the United States and the heat processing of the flaxseed inactivates the potential for hydrogen cyanide production. There is no reasonable toxicological basis to expect a health concern from the use of either defatted or untreated flaxseed in baked goods." The FDA response concluded, "Foods containing up to 12% flaxseed (whether or not the defatted or heat treated) are not deemed unsafe and we wouldn't object to their use" (FDA 1983).

Seven years later, on January 25, 1990, FDA again provided an opinion regarding food use of flaxseed in the U.S. in response to an inquiry from French American Ice Cream Company of Walnut Creek, California (FDA 1990). This response repeated that, "Untreated (non-defatted) flaxseed has been used for food in the U.S. as the heat processing of the flaxseed inactivates the potential for hydrogen cyanide production. There is no reasonable toxicological basis to expect a health concern from the use of defatted or untreated flaxseed in processed foods." After conceding that "FDA does not have data regarding time/temperature requirements needed to inactivate the flaxseed," the response concluded, "Foods containing up to 12% flaxseed (whether or not defatted or heat treated) are not considered to be unsafe and we do not object to their use" (FDA 1990).

Finally, a contribution was provided to the 1995 book *Flaxseed in Human Nutrition* by Dr. John Vanderveen, then-Director of the Office of Plant and Dairy Foods and Beverages at FDA (Vanderveen 1995). In his chapter, Vanderveen (1995) noted that "the regulatory status of flaxseed as an ingredient for human food has not been determined" because no formal petition had been received requesting FDA to evaluate flaxseed as either a food additive or GRAS substance. Nevertheless, "the FDA was aware that defatted flaxseed meal was used for many years as an ingredient in at least two breakfast cereals designed to be served hot and in some specialty breads." The review by Vanderveen (1995) noted an FDA response to Maple Leaf Mills Ltd. of Toronto, Ontario, Canada, in June, 1982, in which FDA concluded, based on both historical information and its own research, that the inclusion of whole untreated flaxseed in bread was safe. Vanderveen (1995) also quoted from an undated FDA response to a Congressional inquiry:

> "On the basis of new information indicating that untreated linseed in uncooked foods such as cold breakfast cereals (containing 10-12 percent linseed) presented no likelihood of any more exposure to hydrogen cyanide than from other foods such as lima beans, fava beans, chickpeas, cassava, yams, cashews or almonds, which also may generate small amounts of hydrogen cyanide during digestion, the import alert was canceled in 1982" (FDA response to a Congressional inquiry, quoted in Vanderveen 1995).

Vanderveen (1995) concluded that "the FDA currently is not challenging the status of flaxseed as an ingredient in bread and other cereal foods," but he cautioned, "the use of flaxseed as a food ingredient does not have food additive status nor has it been affirmed as GRAS. ... Under such circumstances the manufacturer assumes full responsibility for the food's safety."

FDA's lack of concern regarding the safety of flaxseed as a human (or animal) food was further revealed in the agency's response to a biotechnology consultation for a new sulfonylurea-tolerant flaxseed variety, CDC Triffid, also known as line 12115 or FP967 (FDA 1998). FDA noted that, "The developer reports that only the grain is consumed by man or other animals and thus, conducted analytical measures on whole seed." Further, "there are no substantive differences in composition and all results are within the normal range observed in flax." FDA concluded:

> "The developer has concluded, based on its safety and nutritional assessment, that flax modified to be tolerant to sulfonylurea herbicides is not materially different in composition, safety, or any relevant parameter from flaxseed now grown, marketed, and consumed. At this time, based on the developer's description of the data and analysis, the Agency considers the consultation on CDC Triffid flax to be complete."

In reaching this conclusion that genetically modified flaxseed is safe for human consumption, FDA implicitly accepted that non-genetically modified flaxseed is safe for human consumption.

In September of 2006, FDA filed without comment a New Dietary Ingredient notification from Archer Daniel Midlands (ADM) for its dietary supplement ingredient Beneflax™ Flax

Lignan Extract (FDA 2006). This filing constituted a finding by FDA that the notification submitted by ADM provided reasonable assurance that the intended use of Beneflax™ is safe under the terms of the Dietary Supplement Health and Education Act. The intended use of Beneflax™ was described in the notification as the inclusion of the lignan extract in hard capsules at a level of 86 to 860 mg Beneflax™/capsule, providing 30 to 300 mg SDG/capsule. Further, the recommended dosage was stated to be 1 to 4 capsules per day. Thus, FDA accepted as safe an exposure to SDG potentially as high as 1200 mg/day.

As was discussed earlier, different analyses of the SDG content of flaxseed have determined a range of values, from 3.13 mg/g (Smeds et al. 2007) to 13.3 mg/g (Johnsson et al. 2000), with the estimated SDG content being dependent on cultivar, growing conditions, extraction method, and analytical method. Using these estimates, the amount of flaxseed that would need to be ingested in order to obtain 1200 mg of SDG is from 90 g to 383 g. These levels are far higher than those achievable from the intended use of milled or whole flaxseed as food ingredients; as noted earlier (Section 3.5), the estimated daily intake (EDI) of flaxseed from its intended use as a food ingredient is only 16 g.

5.3. Other Authoritative Opinions on the Safety of Flaxseed Products

The genetically modified strain of flaxseed discussed above was also reviewed and approved by Health Canada (1999b). In its review, Health Canada stated:

> "The human consumption of flaxseed from CDC Triffid – FP967 will be mostly via specialty baked goods such as flax/multigrain breads. Additionally, smaller amounts will be made available for human consumption as either milled flaxseed or as flaxseed oil. Flax consumption in Canada is small and direct human consumption has been estimated by the Flax Council of Canada to be on the order of 4,000 tons annually. The genetic modification of CDC Triffid – FP967 will not result in any change in the consumption pattern for flaxseed. Consequently, the dietary exposure of Canadians to this product is anticipated to be the same as for other lines of commercially available flaxseed."

Like FDA, Health Canada (1999b) agreed that the composition of the genetically modified strain did not differ from the parent strain in any ways that would be reasonably expected to affect safety, and thus concluded, "Health Canada is of the opinion that flaxseed from CDC Triffid – FP967 is as safe and nutritious as that available from current commercial flax varieties."

According to the Flax Council of Canada, "No regulation exists that limits the level of flaxseed in foods. The Health Protection Branch of Health Canada has established guidelines for the use of flax in foods at the level of 8% or lower on a dry-weight basis in baking or 4% in dry cereals" (Morris 2003).

Flaxseed products were also reviewed by the Natural Standard Research Collaboration for their use as complementary or alternative medicines for therapeutic use (Basch et al. 2007).

Based on what the reviewers regarded as an inadequate database to evaluate the safety of long-term use of flaxseed or flaxseed oil as therapeutic agents, they concluded that such use for up to 4 months is "likely safe," while use for more than 4 months was regarded as "possibly safe." At the same time, the reviewers conceded that:

> "In the few human case reports available, flaxseed and flaxseed oil appear to be well tolerated. Although limited human safety data exist, there is long-standing historical use with few reports of adverse events" (Basch et al. 2007).

It is important to note that the Natural Standard Research Collaboration's reviews are limited to assessments of the safety and efficacy of substances for use as complementary or alternative medicine. Such use of flaxseed, as was reflected in the human studies discussed earlier, most often involves far higher doses than result from its use as a food ingredient. Few human studies have investigated doses of less than 30 g/day, and in many studies the participants have received doses of 55 g or 60 g/day or even higher. As was discussed in Section 3.5, the estimated daily intake of flaxseed from its intended use as a food ingredient to be added at levels limited only by cGMP is 16 g, about half of the lowest dose suggested as appropriate for therapeutic use.

5.4. Regulatory Framework

The regulatory framework for establishing whether the intended use of a substance is GRAS, in accordance with Section 201(s) of the Federal Food Drug and Cosmetic Act, is set forth under 21 CFR §170.30. This regulation states that general recognition of safety may be based on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. A GRAS determination may be made either: 1) through scientific procedures under §170.30(b); or 2) through experience based on common use in food, in the case of a substance used in food prior to January 1, 1958, under §170.30(c). This GRAS determination employs scientific procedures established under §170.30(b).

A scientific procedures GRAS determination requires the same quantity and quality of scientific evidence as is needed to obtain approval of the substance as a food additive. In addition to requiring scientific evidence of safety, a GRAS determination also requires that this scientific evidence of safety be generally known and accepted among qualified scientific experts. This "common knowledge" element of a GRAS determination consists of two components:

1) 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 experts about the safety of the substance for its intended use.

The criteria outlined above for a scientific-procedures GRAS determination are applied below in an analysis of whether the addition of flaxseed to food as a nutrient supplement to increase the daily intake of total fiber and ALA is safe and is GRAS.

A scientific procedures GRAS determination requires that information about the substance establish that the intended uses of the substance are safe. The FDA has defined "safe" or "safety" for food additives under 21 CFR §170.3(i) as "a reasonable certainty in the minds of competent scientists that the substance is not harmful under its intended conditions of use." This same regulation specifies that three factors must be considered in determining safety. These three factors are:

1) The probable consumption of the substance and of any substance formed in or on food because of its use (i.e., the EDI);

2) The cumulative effect of the substance in the diet, taking into account any chemically- or pharmacologically-related substance or substances in such diet; and

3) Safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients, are generally recognized as appropriate.

5.5. Establishing the Safety of the Intended Use of Flaxseed

5.5.1. EDI of Flaxseed, ALA, and Fiber

Milled and whole flaxseed are intended to be added to conventional foods at levels limited only by cGMP, standards of identity, or other legal constraints.

As was discussed earlier, developing a reasonable estimate of the intake of flaxseed and its constituents likely to result from its addition to conventional foods requires a different approach than is often used for estimating exposure to food additives, pesticide residues, or environmental contaminants. The accepted method for such exposure estimates is to assume that all target foods contain the additive or other substance and multiply its concentration by the amount of the foods consumed. However, flaxseed is an ingredient for which use is constrained by regulatory, technological, and organoleptic limitations. As a nutrient supplement, flaxseed must be used in compliance with FDA's Fortification Policy as presented in 21 CFR §104.20. FDA's statement of purpose for the Fortification Policy indicates that its intention is to "establish a uniform set of principles that will serve as a model for the rational addition of nutrients to foods" (§104.20(a)). Specific policy guidelines include:

- It is not appropriate to fortify fresh produce; meat, poultry, or fish products; sugars; or snack foods such as candies and carbonated beverages (§104.20(a)).

- The food must be suitable to act as a vehicle for the added nutrient (§104.20(b)(1)).

- The food is not the subject of any other Federal requirement ... that prohibits nutrient additions (§104.20(b)(2)).

- A nutrient(s) may appropriately be added to a food that replaces traditional food in the diet to avoid nutritional inferiority (§104.20(e)).

- A nutrient added to a food is appropriate only when the nutrient is stable in the food under customary conditions of storage, distribution, and use (§104.20(g)(1)).

- A nutrient added to a food is appropriate only when the nutrient is physiologically available from the food (§104.20(g)(2)).

- A nutrient added to a food is appropriate only when the nutrient is present at a level at which there is a reasonable assurance that consumption of the food containing the added nutrient will not result in an excessive intake of the nutrient, considering cumulative amounts from other sources in the diet (§104.20(g)(3)).

- A nutrient added to a food is appropriate only when the nutrient is suitable for its intended purpose and is in compliance with applicable provisions of the act and regulations governing the safety of substances in food (§104.20(g)(4)).

The first guideline, in particular, restricts the use of flaxseed as a nutrient supplement by prohibiting its addition to meat, poultry, or fish products, or to foods of limited nutritional value (the so-called "jellybean rule"), although this does not restrict its addition a relatively low levels for technological functions such as binding, thickening, or stabilizing. Flaxseed is also subject to technological and organoleptic limitations: there are many foods for which it is not possible to add flaxseed or any fiber at a high level and still have a product acceptable to consumers.

As was discussed in Section 3.5, FDA has developed an approach to estimating how many servings of foods containing oat fiber or fiber from psyllium husks might reasonably be consumed in a day. FDA concluded that it is not reasonable to expect people to consume more than four servings a day of such foods—one at each meal and a fourth serving at a snack. Applying this same reasoning to flaxseed, it is reasonable to estimate that people will not generally consume more than four servings a day of foods containing this source of fiber. If each of these four servings is of a food contained 4 g of flaxseed, then the daily intake of flaxseed would be 16 g. If some of the foods selected contain fewer than 4 g flaxseed/serving, then the daily intake would be less than 16 g.

Thus, the EDI of flaxseed is 16 g/day. Since flaxseed comprises about 25% α-linolenic acid (ALA) and 25% fiber , the EDI of both ALA and fiber from the intended use of flaxseed is 4 g/day. Since ALA is an essential n-3 fatty acid, since it is widely recognized that increasing the ratio of n-3/n-6 fatty acids in the diet would be beneficial, and since there is no known safety issue with such an increase in ALA intake, such an increase is safe. As discussed previously, for most individuals in the U.S., addition of 4 g/day of fiber merely serves to raise their intake of fiber in the direction of recommended levels, with no likelihood of overconsumption. Further, as was pointed out by the IOM Panel on Macronutrients, the bulking effect of fiber is likely to make consumption self-limiting, again reducing the likelihood of overconsumption. In addition to observing the self-limiting nature of fiber consumption, the IOM Panel determined that there is no likelihood of adverse consequences of excessive fiber consumption other than transient

gastrointestinal disturbances such as minor bloating or flatulence. It was for these reasons that the panel concluded that there was no basis for setting a Tolerable Upper Intake Level for either dietary fiber or functional fiber.

5.5.2. History of Consumption of Flaxseed

Vaisey-Genser and Morris (2003) reported that:

> "Humans have been eating flax for thousands of years. Flax is a founding crop, being one of the first domesticated plants. Its cultivation likely began in the fertile valleys of the so-called Fertile Crescent in Mesopotamia about 8,000 to 10,000 years ago. Flax was valued in Ancient and Early Modern times as both a food and medicine."

Historical records indicate that flaxseed was consumed in Turkey, Syria, Jordan, and Iran at least as early as 9000-8000 B.C. (Hall et al. 2006).

Writing for the Canadian Department of Agriculture and Agri-Food, Oomah (2001) observed that, "Flaxseed consumption in various forms as a food ingredient and for its medicinal properties dates from 5000 BC since its cultivation." Another Canadian government scientist from the Health Products and Food Branch of Health Canada wrote, "It is common, at least in Canada, to find bread and other bakery products with flaxseed added" (Lee 2006).

Similarly, FDA scientists Babu et al. (2003) stated that, "Flaxseed has a history of food use in Europe and Asia. At about 12% level, flaxseed has been accepted as a component in some brands of cereal, in specialty breads and cookies, as a seed dressing, and in other bakery products in the U.S. Food products containing flaxseed are found primarily in the north and midwestern parts of the United States." In the same year, the same group of FDA scientists also wrote, "Flaxseed food products have been found with increasing frequency in supermarkets and health food stores all over the country. Increased consumption of flaxseed products by everyone, including pregnant women, is anticipated due to [its] potential health benefits" (Collins et al. 2003). As was discussed previously, the Natural Standard Research Collaboration's review of flaxseed (for use as a complementary or alternative medicine) concluded that, "Although limited human safety data exist, there is long-standing historical use with few reports of adverse events" (Basch et al. 2007).

Despite its long history of food use, flaxseed has remained a minor constituent of the diet in the United States, and it therefore is not recorded in the national food consumption surveys; thus, current consumption of flaxseed cannot be quantitatively documented. As a part of its New Dietary Ingredient notification to FDA, Archer Daniel Midlands included a listing of some food products containing flaxseed (ADM 2006). These included:

- Country Harvest Flax Bagels
- Flax Plus Raisin Bran Cereal
- Flax Plus Multibran Cereal

- Stonemill Flax & Honey Bagels
- Stonemill Golden Flax Bread
- Country Harvest Sunflower Flax Bread
- Casa Mendosa Tortillas
- Lavash Flatbread Pita Bread
- Country Harvest Flax Bread
- Nature's Path Instant Flax 'n Oats (hot oatmeal cereal)
- Nature's Path Multibran Cereal
- Golden Flax High Fiber Cereal

This same notification also reported data from a Mintel Global New Products Database of new flaxseed product introductions in 2004 (ADM 2006). According to these data, 166 flaxseed products were introduced in the U.S. that year, along with 57 in Canada, 6 each in the UK and France, and smaller numbers in countries ranging from Australia to Israel.

5.5.3. Toxicity Studies And Human Trials Establishing the Safety of Flaxseed

In addition to the presumption of safety of the intended use of flaxseed resulting from its history of use, there is abundant experimental evidence available to establish the safety of the intake of flaxseed anticipated to result from its intended use. This evidence includes extensive oral toxicity studies of flaxseed and its constituents in rats, mice, piglets and rabbits as well as numerous human trials in both healthy and diseased populations in which the only effect observed that might be regarded as adverse was increased laxation, observed in a small number of studies.

Far from producing adverse effects, flaxseed appears to provide several physiological benefits. The most firmly established benefit resulting from ingestion of flaxseed fiber is increased laxation; attenuation of the postprandial rise in insulin and/or glucose levels and beneficial effects on blood lipids are supported by some experimental evidence but must be regarded as unproven. Other benefits that have been studied in a small number of studies and received some support include reductions in the risk of the incidence or progression of prostate or breast cancer, anti-inflammatory properties (most likely attributable to ALA), and renoprotective effects, especially in lupus nephritis patients. The evidence for these benefits must be regarded as no more than suggestive.

Both flaxseed itself and is constituents, including flaxseed meal (defatted flaxseed), flaxseed oil, and lignans, have been subjected to toxicological assessment in a number of subchronic toxicity studies ranging in duration from 50 days to 125 days. The no observed adverse effect level (NOAEL) for flaxseed was in all cases the highest level tested in both the rat and the piglet:

- 40% dietary concentration (30,000 mg/kg bw/day) in the SD rat for 90 days (Raynayake et al. 1992)

- 10% dietary concentration (7500 mg/kg bw/day) in the SD rat for 50 days (Yuan et al. 1999)

- 10% dietary concentration (7500 mg/kg bw/day) in the Fischer 344 rat for 100 days (Hemmings et al. 2004)

- 10% dietary concentration (2700 mg/kg bw/day) in the Yorkshire x Landrace gilt (piglet) for 125 days (Farmer et al. 2007)

The NOAEL for flaxseed meal was also the highest level tested, 6.5% dietary concentration (1750 mg/kg bw/day) in the Yorkshire x Landrace gilt (piglet) for 125 days (Farmer et al. 2007), and the NOAEL for flaxseed oil was the highest level tested, 3.5% dietary concentration (950 mg/kg bw/day) in the same study. The NOAEL for flaxseed oil in the rat, however, was 1950 mg/kg bw/day, based on the findings of Hemmings et al. (2004).

Three toxicity studies of secoisolariciresinol diglucoside (SDG) both determined that the NOAEL was the only dose tested:

- 9 mg/kg bw/day of SDG by gavage in the SD rat for 50 days (Yuan et al. 1999)

- 63 mg/kg bw/day of SDG in the diet of the Fischer 344 rat for 100 days (Hemmings et al. 2004)

- 40 mg/kg bw/day of flaxseed lignan complex (containing 14 mg/kg bw/day of SDG) in the diet of the New Zealand white rabbit for 60 days (Prasad 2005)

The only subchronic toxicity study relevant to flaxseed safety in which the NOAEL was not the highest dose tested was a 90-day study in which Wistar rats received up to 4% dietary concentration of 7-hydroxymatairesinol (7-HMR), a lignan found only in trace quantities in flaxseed but chemically related to matairesinol (Lina et al. 2005). The NOAEL in this study was 0.25% dietary concentration, equivalent to 160 mg/kg bw/day.

Because of the potential for estrogenic effects of flaxseed, particularly due to the lignan content, there have been a large number of toxicity studies addressing developmental and reproductive parameters. The 15 published studies available have assessed the safety of flaxseed or a constituent—flaxseed meal, ALA, or SDG. All but one of these studies used rat models, nearly all of them Sprague-Dawley rats, and one study of flaxseed oil was conducted with Sv/Ev mice. The flaxseed doses used in these studies ranged from 5% dietary concentration (approximately 3750 mg/kg bw/day) to 40% dietary concentration (30,000 mg/kg bw/day).

Studies in which flaxseed was administered at 5% dietary concentration most often found no effect ((Tou and Thompson 1997; Orcheson et al. 1998; Tou et al. 1998b) as did a number of studies that tested higher doses: no effect was found with flaxseed at 10% of the diet by Orcheson et al. (1998), Ward et al. (2001a), Ward et al. (2001b), or Hemmings and Barker (2004). An absence of effects has been observed at even higher doses; Wiesenfeld et al. (2003) found no effects other than non-adverse changes in fatty acid absorption in SD rats given

flaxseed meal (defatted flaxseed) at 13% or 26% of the diet or flaxseed at 20% or 40% of the diet. Other studies assessing specific adverse effects have also found an absence of adverse effects from flaxseed even at high concentrations; Sprando et al. (2000b) found no effect of flaxseed on spermatogenesis or testis structure at either 20% or 40% of the diet.

However, other studies of higher dietary concentrations of flaxseed have found estrogenic or antiestrogenic effects. These effects often appear to be contradictory, with one effect appearing at one dose and the opposite effect appearing at a different dose, particularly in the studies by Tou et al., who in 1999 (Tou et al. 1999) suggested that the dose, timing, and duration of flaxseed exposure all affect its hormonal effects and concluded that lower concentrations tend to produce antiestrogenic effects while higher concentrations produce estrogenic effects. Although no adverse effects were seen on growth, clinical chemistry, hematology, or histopathology at any dose, flaxseed appears to consistently produce weak estrogenic effects at high levels of intake even though these effects seem to be inconsistent from study to study. Although Wiesenfeld et al. (2003) found no adverse effects in dietary concentrations as high as 40%, approximately equivalent to 30,000 mg/kg bw/day, other investigators did see effects at this level that may be regarded as adverse.

The totality of the research appears to support the conclusion that consistent estrogenic effects are not observed at doses of flaxseed as high as 3750 mg/kg bw/day (5% dietary concentration). It is also notable that human studies in which flaxseed was fed at high doses have generally found an absence of estrogenic effects. Phipps et al. (1993) gave women 10 g/day of flaxseed (170 mg/kg bw/day for a 60-kg woman) through 7 complete menstrual cycles and observed no estrogenic or antiestrogenic effects. Lucas et al. (2002) administered flaxseed to postmenopausal women for 3 months at even higher doses, 40 g/day (670 mg/kg bw/day for a 60-kg woman), and found no estrogenic effects on sex hormones.

Since the EDI of flaxseed from its intended use as a food ingredient is 16 g/day, only a tiny fraction of the amount that has been shown to be without harm in a large number of animal toxicity studies and human studies, it may be concluded that there is a reasonable certainty of no harm from the intended addition of flaxseed to food limited only by cGMP.

5.6. General Recognition of the Safety of Whole and Milled Flaxseed

The intended addition of flaxseed to food has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b). This safety was established by first establishing the identity and purity of the material, estimating potential human exposure to flaxseed from its intended use, and demonstrating that this level of exposure is without significant risk of harm. Finally, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of the intended use of whole and milled flaxseed has been made through the deliberations of an Expert Panel consisting of Joseph F. Borzelleca, Ph.D., Walter H. Glinsmann, M.D., Robert J. Nicolosi, Ph.D., and John A. Thomas, Ph.D. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have critically reviewed and evaluated the publicly

available information summarized in this document, including the potential human intake resulting from the intended uses of flaxseed, as well as other information available to them, and have individually and collectively concluded:

> *Both whole and milled flaxseed have been sufficiently characterized to ensure that they are food-grade products and that no toxicity concerns from impurities exist. Ingestion of flaxseed from its intended use results in levels of intake of flaxseed and its constituents that are within safe limits established by the history of consumption of flaxseed and by published animal toxicity studies and human studies of flaxseed and its constituents. Therefore, the intended use of whole and milled flaxseed, produced and used in accordance with cGMP and complying with the specifications described in the GRAS monograph is safe and Generally Recognized As Safe (GRAS) based on scientific procedures and supported by a history of use..*

It is the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same conclusion.

6. LITERATURE CITED

Adlercreutz H, K Hockerstedt, C Bannwart, S Bloigu, E Hamalainen, T Fotsis, A Ollus. 1987. Effect of dietary components, including lignans and phytoestrogens on enterohepatic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG). J Steroid Biochem Mol Biol 27:1135-1144.

AmeriFlax. 2008. Flaxseed information. Available online at http://www.ameriflax.com.

Anderson E and HJ Lowe. 1947. The composition of flaxseed mucilage. J Biol Chem 168:289-297.

Andersson SO, A Wolk, R Bergstrom, E Giovannucci, C Lindgren, J Baron, HO Adami. 1996. Energy, nutrient intake and prostate cancer risk: a population –based case-control study in Sweden. Int J Cancer 68:716-722.

Archer Daniel Midlands (ADM). 2006. New Dietary Ingredient notification for Beneflax™ Flax Lignan Extract. Submitted to FDA on June 6.

Arjmandi BH, DA Khan, S Juma, ML Drum, S Venkatesh, E Sohn, L Wei, R Derman. 1998. Whole flaxseed consumption lowers serum LDL-cholesterol and lipoprotein(a) concentrations in postmenopausal women. Nutr Res 18:1203-1214.3

Babu US, PW Wiesenfeld, TFX Collins, R Sprando, TJ Flynn, T Black, N Olejnik, RB Raybourne. 2003. Impact of high flaxseed diet on mitogen-induced proliferation, IL-2 production, cell subsets and fatty acid composition of spleen cells from pregnant and F_1 generation Sprague-Dawley rats. Food Chem Toxicol 41:905-915.

Bacala R and VJ Barhtet. 2007. Development of extraction and gas chromatography analytical methodology for cyanogenic glycosides in flaxseed (*Linum usitatissimum*). J AOAC Int 90:153-161.

Bankovic-Calic N, MR Ogbori, E Nicman. 2002. Effect of a modified low protein and low fat diet on histologic changes and metabolism in kidneys in an experimental model of polycystic kidney disease. Srp Arh Celok Lek 130:251-257. [Article in Serbian with English abstract]

Barthet VJ. 2006. *Measure of cyanogenic glycosides in flaxseed by HPLC and rapid glucose or CN analysis*. Winnipeg: Flax Council of Canada; R04-13.

Barthet V and J Daun. 2000. *Sources, metabolism and toxicity of cyanide and the cyanogenic glycosides*. Unpublished report prepared for the Flax Council of Canada.

Basch E, S Bent, J Collins, C Dacey, P Hammerness, M Harrison, M Smith, P Szapary, C Ulbricht, M Vora, W Weissner. 2007. Flax and flaxseed oil (*Linum usitatissimum*): a review by the Natural Standard Research Collaboration. J Soc Integrated Oncol 5:92-105.

000156

Bean LD and S Leeson. 2002. Fatty acid profiles of 23 samples of flaxseed collected from commercial feed mills in Ontario in 2001. J Appl Poult Res 11:209-211.

Bhathena SJ, AA Ali, C Haudenschild, P Latham, T Ranich, AI Mohamed, CT Hansen, MT Velasquez. 2003. Dietary flaxseed meal is more protective than soy protein concentrate against hypertriglyceridemia and steatosis of the liver in an animal model of obesity. J Am Coll Nutr 22:157-164.

Bhatty RS. 1993. Further compositional analyses of flax: musilage, trypsin inhibitors and hydrocyanic acid. J Am Oil Chemists' Soc 70:899-904.

Bierenbaum ML, R Reichstein, TR Watkins. 1993. Reducing atherogenic risk in hyperlipemic humans with flaxseed supplementation: a preliminary report. J Am Coll Nutr 12:501-504.

Bretillon L, JM Chardigny, JL Sebedio, JP Noel, CM Scrimgeour, CE Fernie, O Loreau, P Gachon, B Beaufrere. 2001. Isomerization increases the postprandial oxidation of linoleic acid but not α-linolenic acid in mean. J Lipid Res 42:995-997.

Brooks JD, WE Ward, JE Lewis, J Hilditch, L Nickell, E Wong, LU Thompson. 2004. Supplementation with flaxseed alters estrogen metabolism in postmenopausal women to a greater extent than does supplementation with an equal amount of soy. Am J Clin Nutr 79:318-325.

Brouwer IA, MB Katan, PL Zock. 2004. Dietary α-linolenic acid is associated with reduced risk of fatal coronary heart disease, but increased prostate cancer risk: a meta-analysis. J Nutr 134:919-922.

Burdge GC. 2004. Alpha-linolenic acid metabolism in men and women: Nutritional and biological implications. Curr Opin Clin Nutr Metab Care 7:137-144.

Burdge GC. 2006. Metabolism of α-linolenic acid in humans. Prostaglandins, Leukot Essent Fatty Acids 75:161-168.

Burdge GC and SA Wootton. 2002. Conversion of α-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. Br J Nutr 88:411-420.

Burdge GC and SA Wootton. 2003. Conversion of α-linolenic acid to palmitic, palmitoleic, stearic and oleic acids in men and women. Prostaglandins, Leukot Essent Fatty Acids 69:283-290.

Burdge GC and PC Calder. 2005. Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. Reprod Nutr Dev 45:581-597.

Burdge GC, AE Jones, SA Wootton. 2002. Eicosapentaenoic and docosapentaenoic acids are the principal products of α-linolenic acid metabolism in young men. Br J Nutr 88:355-363.

Burdge GC, YE Finnegan, AM Minihane, CM Williams, SA Wootton. 2003. Effect of altered dietary n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [^{13}C] α-linolenic acid to longer-chain fatty acids and partitioning towards β-oxidation in older men. Br J Nutr 90:311-321.

Butler GW and EE Conn. 1964. Biosynthesis of cyanogenic glucosides. I. Labeling studies *in vivo* with *Linum usitatissimum*. J Bil Chem 239:1674-1679.

Canvin DT. 1965. The effect of temperature on the oil content and fatty acid composition of the oils from several oil seed crops. Can J Bot 43:63-69.

Chadha RK, JF Lawrence, WMN Ratnayake. 1995. Ion chromatographic determination of cyanide released from flaxseed under autohydrolysis conditions. Food Addit Contam 12:527-533.

Chaney RL, JA Ryan, Y-M Li, LS Angle. 2001. Transfer of Cadmium Through Plants to the Food Chain, In *Proceedings Workshop Environmental Cadmium in the Food Chain: Sources, Pathways, and and Risks*, Scientific committee on Problems of the Environment, Paris, 76-81.

Chen Y, E Kenaschuk, P Dribnenki. 1999. Response of flax genotypes to doubled haploid production. Plant Cell Tiss Org Cult 57:195-198.

Clark WF, A Parbtani, MW Huff, E Spanner, H de Salis, I Chin-Yee, DJ Philbrick, BJ Holub. 1995. Flaxseed: a potential treatment for lupus nephritis. Kidney Inter 48:475-480.

Clark WF, C Kortas, AP Heidenheim, J Garland, E Spanner, A Parbtani. 2001. Flaxseed in lupus nephritis: a two-year nonplacebo-controlled crossover study. J Am Coll Nutr 20:143-148.

Cohen SL and WE Ward. 2005. Flaxseed oil and bone development in growing male and female mice. J Toxicol Environment Health Part A 68:1861-1870.

Collins TFX, RL Sprando, TN Black, N Olejnik, PW Wiesenfeld, US Babu, M Bryant, TJ Flynn, DI Ruggles. 2003. Effects of flaxseed and defatted flaxseed meal on reproduction and development in rats. Food Chem Toxicol 41:819-834.

Cunnane SC, S Ganguli, C Menard, AC Liede, MJ Hamadeh, Z-Y Chen, TMS Wolever, DJA Jenkins. 1993. High α-lenolenic acid flaxseed (*Linum usitatissimum*): some nutritional properties in humans. Br J Nutr 69:443-453.

Cunnane SC, MJ Hamadeh, AC Liede, LU Thompson, TMS Wolever, DJA Jenkins. 1994. Nutritional attributes of traditional flaxseed in healthy young adults. Am J Clin Nutr 61:62-68.

Dahl WJ, EA Lockert, AJ Cammer, SJ Whiting. 2005. Effects of flax fiber on laxation and glycemic response in healthy volunteers. J Med Food 8:508-511.

Daun JK. 2008a. Cyanogenic glycoside and potential cyanide content of flaxseed. Unpublished report to Flax Canada 2015 Inc.

Daun JK. 2008b. Effect of Processing on the Cyanogenic Glycoside Content of Flaxseed. Unpublished report to Flax Canada 2015 Inc. dated March 4, 2008.

Daun JK and R Przybylski. 2000a. Effect of environment as determined by oil content and unsaturation of the composition of flaxseed, INFORM Abstracts, 91st AOCS Annual Meeting & Expo, San Diego, CA. April 25-28. 11:106.

Daun JK and R Przybylski. 2000b. Environmental effects on the composition of four Canadian flax cultivars. In *Proceedings of the 58th Flax Institute of the United States*; Carter J; Flax Institute of the United States: Fargo ND; 80-91.

Daun JK and R Thomas. 2005. Fat content of oilseeds used as foods is dependent on the method of determination. JAOCS 82:767-769.

Daun JK, VJ Barthet, TL Chornick, S Duguid. 2003. Chapter 1. Structure, composition, and variety development of flaxseed. In *Flaxseed in Human Nutrition*, 2nd Edition; LU Thompson and SC Cunnane (Eds.) AOCS Press: Champaign, Illinois.

DeClercq DR. 2008. *Quality of Flaxseed in Canada*. Canadian Grain Commission: On-line report available at http://www.grainscanada.gc.ca

DeLany JP, MM Windhouser, CM Champagne, GA Bray. 2000. Differential oxidation of individual dietary fatty acids in humans. Am J Clin Nutr 72:905-911.

Demark-Wahnefried W, DT Price, TJ Polascik, CN Robertson, EE Anderson, DF Paulson, PJ Walther, M Gannon, RT Vollmer. 2001. Pilot study of dietary fat restriction and flaxseed supplementation in men with prostate cancer before surgery: exploring the effects on hormonal levels, prostate-specific antigen, and histopathologic features. Urol 58:47-52.

De Stefani E, H Deneo-Pellegrini, P Boffetta, A Ronco, M Mendilaharsu. 2000. α-linolenic acid and risk of prostate cancer: a case-control study in Uruguay. Cancer Epidemiol Biomark Prev 9:335-338.

Diederichsen A and K Richards. 2003. Cultivated flax and the genus *Linum* L. Chapter in AD Muir and ND Westcott, Eds., *Flax: the genus Linum*. London: Routledge.

Dodin S, A Lemay, H Jacques, F Legare, J-C Forest, B Masse. 2005. The effects of flaxseed dietary supplement on lipid profile, bone mineral density, and symptoms in menopausal women: a randomized, double-blind, wheat germ placebo-controlled clinical trial. J Clin Endocrinol Metab 90:1390-1397.

Duguid SD. 2007. Report of flax co-operative test 2007. Unpublished report conducted for The Prairie Recommending Committee for Oilseeds.

000159

Dybing CD and DC Zimmerman. 1966. Fatty acid accumulation in maturing flaxseeds as influenced by environment. Plant Physiol 41:1463-1470.

Eckey EW. 1954. *Vegetable Fats and Oils.* New York: Reinhold, p 541.

Evans BAJ, K Griffiths, MS Morton. 1995. Inhibition of 5-alpha reductase in genital skin fibroblasts and prostate tissue by dietary lignans and isoflavonoids. J Endocrinol 147:295-302.

Faintuch J, LM Horie, HV Barbeiro, DF Barbeiro, FG Soriano, RK Ishida, I Cecconello. 2007. Systemic inflammation in morbidly obese subjects: response to oral supplementation with alpha-linolenic acid. Obesity Surg 17:341-347.

Farmer C, HV Petit, H Weiler, AV Capuco. 2007. Effects of dietary supplementation with flax during prepuberty on fatty acid profile, mammogenesis, and bone resorption in gilts. J Anim Sci 85:1675-1686.

Feng D, Y Shen, ER Chavez. 2003. Effectiveness of different processing methods in reducing hydrogen cyanide content of flaxseed. J Sci Food Agric 83:836-841.

Flax Council. 2008. *Growth and Development/Harvesting.* Flax Council of Canada website: http://www.flaxcouncil.ca.

Flynn TJ, TFX Collins, RL Sprando, TN Black, DI Ruggles, PW Wiesenfeld, US Babu. 2003. Developmental effects of serum from flaxseed-fed rats on cultured rat embryos. Food Chem Toxicol 41:835-840.

Food and Drug Administration (FDA). 1983. Letter to Harvest Foods Ltd of Saskatoon, Saskatchewan, Canada, from Division of Regulatory Guidance, Bureau of Foods, FDA, February 7.

Food and Drug Administration (FDA). 1990. Letter to French American Ice Cream Company of Walnut Creek, California, from the Minneapolis District, FDA, January 25.

Food and Drug Administration (FDA). 1997a. Food labeling: health claims; oats and coronary heart disease; final rule. Federal Register 62:3583-3601.

Food and Drug Administration (FDA). 1997b. Substances affirmed as generally recognized as safe: menhaden oil; final rule. Federal Register 62:30751.

Food and Drug Administration (FDA). 1998. Biotechnology consultation, note to the file: BNF No. 000050 (sulfonylurea tolerant linseed flax). March 24.

Food and Drug Administration (FDA). 2006. Letter from the Office of Nutritional Products, Labeling and Dietary Supplements to the Dockets Management Branch requesting the filing of New Dietary Ingredient notification Rpt. 355, BeneFlax™ Flax Lignan Extract," September 14.

Frehner M, M Scalet, EE Conn. 1990. Pattern of the cyanide-potential in developing fruits. Plant Physiol. 94:28-34.

Frische EJ, AM Hutchins, MC Martini, W Thomas, JL Slavin. 2003. Effect of flaxseed and wheat bran on serum hormones and lignan excretion in premenopausal women. J Am Coll Nutr 22:550-554.

Fuchs D, R Piller, J Linseisen, H Daniel, U Wenzel. 2007. The human peripheral blood mononuclear cell proteome responds to a dietary flaxseed intervention and proteins identified suggest a protective effect in atherosclerosis. Proteomics 7:3278-3288.

Gann PH, CH Hennekens, FM Sacks, F Grodstein, EL Giovannucci, MJ Stampfer. 1994. Prospective study of plasma fatty acids and risk of prostate cancer. J Natl Cancer Inst 86:281-286.

Gebauer SK, TL Psota, WS Harris, PM Kris-Etherton. 2006. n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. Am J Clin Nutr 83(6 Suppl):1526S-1535S.

Giovannucci E, EB Rimm, GA Colditz, MJ Stampfer, A Ascherio, CC Chute, WC Willett. 1993. A prospective study of dietary fat and risk of prostate cancer. J Natl Cancer Inst 85:1571-1579.

Godley PA, MK Campbell, C Miller, P Gallagher, FE Martinson, JL Mohler, RS Sandler. 1996. Correlation between biomarkers of omega-3 fatty acid consumption and questionnaire data in African American and Causcasian United States males with and without prostatic carcinoma. Cancer Epidemiol Biomark Prev 5:115-119.

Grant CA and LD Bailey. 1997. Effects of phosphorus and zinc fertilizer management on cadmium accumulation in flaxseed. J Sci Food Agric 73:307-314.

Grant CA, JCP Dribnenki, LD Bailey. 2000. Cadmium and zinc concentrations and ratios in seed and tissue of solin (cv Linola-T-M 947) and flax (cvs McGregor and Vimy) as affected by nitrogen and phosphorus fertilizer and provide (*Penicillium bilaji*). J Sci Food Agric 80:1735-1743.

Griel AE, PM Kris-Etherton, KF Hilpert, G Zhao, SG West, RL Corwin. 2007. An increase in dietary n-3 fatty acids decreases a marker of bone resorption in humans. Nutr J 6:2.

Haggans CJ, AM Hutchins, BA Olson, W Thomas, MC Martini, JL Slavin. 1999. Effect of flaxseed consumption on urinary estrogen metabolites in postmenopausal women. Nutr Cancer 33:188-195.

Haggans CJ, EJ Travelli, W Thomas, MC Martini, JL Slavin. 2000. The effect of flaxseed and wheat bran consumption on urinary estrogen metabolites in premenopausal women. Cancer Epidemiol 9:719-725.

Hall C, MC Tulbek, Y Xu. 2006. *Flaxseed. Advances in food and nutrition research*, Vol 51. Academic Press.

Hallund J, G Ravn-Haren, S Bugel, T Tholstrup, I Tetens. 2006. A lignan complex isolated from flaxseed does not affect plasma lipid concentrations or antioxidant capacity in healthy postmenopausal women. J Nutr 136:112-116.

Hammond JJ, JF Filler, CE Green, RL Chaney. 2000. Screening the USDA Flax Collection for Seed Cadmium, In *Proceedings of the 58th Flax Institute of the United States*, Fargo, North Dakota, pp. 108-115.

Haque MR and JH Bradbury. 2002. Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods. Food Chem 77:107-114.

Harvei S, KS Bjerve, S Tretti, E Jellum, TE Robsahm, L Vatten. 1997. Prediagnostic level of fatty acids in serum phospholipids: omega-3 and omega-6 fatty acids and the risk of prostate cancer. Int J Cancer 71:545-551.

Health Canada. 1988. *Guideline concerning the safety and physiological effects of novel fibre sources and food products containing them*. Ottawa: Food Directorate, Health Protection Branch, Health Canada.

Health Canada. 1999a. *Nutrient value of some common foods*. Canadian Government Publishing: Ottawa.

Health Canada. 1999b. Novel food information - food biotechnology: sulfonylurea tolerant flax, CDC Triffid – FP967. Document FD/OFB-098-047-A, October.

Health Canada. 2007. Warfarin interactions with drugs, herbs and food. Available online at http://www.hc-sc.gc.ca/.

Hedelin M, A Klint, ET Chang, R Bellocco, J-E Johansson, S-O Andersson, S-M Heinonen, H Adlercreutz, H-O Adami, H Gronberg, KA Balter. 2006. Dietary phytoestrogen, serum enterolactone and risk of prostate cancer: the Cancer Prostate Sweden Study (Sweden). Cancer Causes Control 17:169-180.

Hemmings SJ and L Barker. 2004. The effects of dietary flaxseed on the Fischer 344 rat: I. Development, behaviour, toxicity and the activity of liver γ-glutamyltranspeptidase. Cell Biochem Funct 22:113-121.

Hemmings SJ, N Westcott, A Muir, D Czechowicz. 2004. The effects of dietary flaxseed on the Fischer 344 rat: II. Liver γ-glutamyltranspeptidase activity. Cell Biochem Funct 22:225-231.

Hocking PJ and MJ McLaughlin. 2000. Genotypic variation in cadmium accumulation by seed of linseed, and comparison with seeds of some other crop species. Austral J Agric Res 51:427-433.

Hodgins ME and CA Patterson. 1994. Potential markets for flax fractions in North America. Report prepared for Saskatchewan Agriculture, Food & Rural Revitalization by Hodgins & Company Management Consultants, Saskatoon, Saskatchewan, Canada.

House SD, PA Larson, RR Hohnson, JW Devries, DL Martin. 1994. Gas chromatographic determination of total fat extreacted from food samples using hydrolysis in the presence of antioxidant. J AOAC Int 77:960-965.

Hutchins AM, MC Martini, BA Olson, W Thomas, JL Slavin. 2000. Flaxseed influences urinary lignan excretion in a dose-dependent manner in postmenopausal women. Cancer Epidemiol 9:1113-1118.

Hyvarinen HK, JM Pihlava, JA Hudenhovi, V Hietaniemi, HJ Korhonen, EL Ryhanen. 2006. Effect of processing and storage on the stability of flaxseed lignan added to bakery products. J Agric Food Chem 54:48-53.

Institute of Medicine (IOM). 2001. *Dietary reference intakes: proposed definition of dietary fiber*. Washington DC: National Academies Press.

Institute of Medicine (IOM). 2002. *Dietary reference intakes: Energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*. Prepublication copy. Washington DC: National Academies Press.

Jenkins DJA, CWC Kendall, E Vidgen, S Agarwal, AV Rao, RS Rosenberg, EP Diamandis, R Novokmet, CC Mehling, T Perera, LC Griffin, SC Cunnane. 1999. Health aspects of partially defatted flaxseed, including effects on serum lipids, oxidative measures, and ex vivo androgen and progestin activity: a controlled crossover trial. Am J Clin Nutr 69:395-402.

Johnsson P, A Kamal-Eldin, LN Lundgren, P Aman. 2000. HPLC method for analysis of secoisolariciresinol diglucoside in flaxseeds. J Agric Food Chem 48:5216-5219.

Joint WHO/FAO Expert Committee on Food Additives (JECFA). 1993. Cyanogenic glycosides. In: *Toxicological evaluation of certain food additives and naturally occurring toxicants*. Geneva, World Health Organization, 39th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (WHO Food Additives Series 30; Available at http://www.inchem.org/documents/jecfa/jecmono/v30je18.htm).

Kaminskas A, B Zieden, B Elving, M Kristenson, A Abaravicius, B Bergdayl, AG Olsson, Z Kucinskiene. 1999. Adipose tissue fatty acids in men from two populations with different cardiovascular risk: the LiVicordia Study. Scand J Clin Lab Invest 59:227-232.

Kelley DS, LB Branch, JE Love, PC Taylor, YM Rivera, JM Iacono. 1991. Dietary alpha-linolenic acid and immunocompetence in humans. Am J Clin Nutr 53:40-46.

Khan G, P Penttinen, A Cabanes, A Foxworth, A Chezek, K Mastropole, B Yu, A Smeds, T Halttunen, C Good, S Makela, L Hilakivi-Clarke. 2007. Maternal flaxseed diet during pregnancy or lactation increases female rat offspring's susceptibility to carcinogen-induced mammary tumorigenesis. Repro Tosicol 23:397-406.

Kinniry P, Y Amrani, A Vachani, CC Solomides, E Arguiri, A Workman, J Carter, M Christofidou-Solomidou. 2006. Dietary flaxseed supplementation ameoiorates inflammation and oxidative tissue damage in experimental models of acute lung injury in mice. J Nutr 136:1545-1551.

Kitts DD, YV Yuan, AN Wijewickreme, LU Thompson. 1999. Antioxidant activity of the flaxseed lignan secoisolariciresinol diglycoside and its mammalian lignan metabolites enterodiol and enterolactone. Mol Cell Biochem 202:91-100.

Klosterman HJ. 1974. Vitamin B_6 antagonists of natural origin. J Agric Food Chem 22:13-16.

Klosterman, HJ, GL Lamoureux, JL Parson. 1967. Isolation, characterization, and synthesis of linatine. A vitamin B_6 antagonist from flaxseed (*Linum usitatissimum*). Biochem 6:170-177.

Kobaisy M. BD Oomah, G Mazza. 1996. Determination of cyanogenic glycosides in flaxseed by barbituric acid-pyridine, pyridine-pyrazolone and high-performance liquid chromatography methods. J Agric Food Chem 44:3178-3181.

Kuijsten A, ICW Arts, P van't Veer, PCH Hollman. 2005a. The relative bioavailability of enterolignans in humans is enhanced by milling and crushing of flaxseed. J Nutr 135:2812-2816.

Kuijsten A, ICW Arts, TB Vree, PCH Hollman. 2005b. Pharmacokinetics of enterolignans in healthy mean and women consuming a single dose of secoisolariciresinol diglucoside. J Nutr 135:795-801.

Kulling SE, E Jacobs, E Pfeiffer, M Metzler. 1998. Studies on the genotoxicity of the mammalian lignans enterolactone and enterodiol and their metabolic precursors at various endpoints in vitro. Mutat Res 416:115-124.

Kurzer MS, JW Lampe, MC Martini, H Adlercreutz. 1995. Fecal lignan and isoflavonoid excretion in premenopausal women consuming flaxseed powder. Cancer Epidemiol 4:353-358.

Lampe JW, MC Martini, MS Kurzer, H Adlercreutz, JL Slavin. 1994. Urinary lignan and isoflavonoid excretion in premenopausal women consuming flaxseed powder. Am J Clin Nutr 60:122-128.

Lampe JW, C Atkinson, MA Hullar. 2006. Assessing exposure to lignans and their metabolites in humans. J Assoc Official Anal Chem 89:1174-1181.

Lee N. 2006. Phytoestrogens as bioactive ingredients in functional foods: Canadian regulatory update. J AOAC 89:1135-1137.

Leitzmann MF, MJ Stampfer, DS Michaud, K Augustsson, GC Colditz, WC Willett, EJ Giovannucci. 2004. Dietary intake of n-3 and n-6 fatty acids and the risk of prostate cancer. Am J Clin Nutr 80:204-216.

Lemay A, S Dodin, N Kadri, H Jacques, J-C Forest. 2002. Flaxseed dietary supplement versus hormone replacement therapy in hypercholesterolemic menopausal women. Obstet Gynecol 100:495-504.

Lewis JE, LA Nickell, LU Thompson, JP Szalai, A Kiss, JR Hilditch. 2006. A randomized controlled trial of the effect of dietary soy and flaxseed muffins on quality of life and hot flashes during menopause. Menopause. 13:631-642.

Lina B, H Korte, L Nyman, M Unkila. 2005. A thirteen week dietary toxicity study with 7-hydroxymatairesinol potassium acetate (HMR lignan) in rats. Regul Toxicol Pharmacol 41:28-38.

Lucas EA, RD Wild, LJ Hammond, DA Khalil, S Juma, BP Daggy, BJ Stoecker, BH Arjmandi. 2002. Flaxseed improves lipid profile without altering biomarkers of bone metabolism in postmenopausal women. J Clin Endocrinol Metab 87:1527-1532.

Malcolmson LJ, R Przybylski, JK Daun. 2000. Storage stability of milled flaxseed. JAOCS 77:235-238.

Mannisto S, P Pietinen, MJ Virtanen, I Salminen, D Albanes, E Giovannucci, J Virtamo. 2003. Fatty acids and risk of prostate cancer in a nested case-control study in male smokers. Cancer Epidemiol Biomarkers Prev 12:1422-1428.

Manthey FA, RE Lee, CA Hall. 2002. Processing and cooking effects on lipid content and stability of α-linolenic acid in spaghetti containing ground flaxseed. J Agric Food Chem 50:1668-1671.

Mazur W, T Fotsis, K Wahala, S Ojala, A Salakka, H Adlercreutz. 1996. Isotope dilution gas chromatographic—mass spectrometric method for the determination of isoflavonoids, coumestrol, and lignans in food samples. Anal Biochem. 233:169-180.

Mazza G and CG Biliaderis. 1989. Functional properties of flax seed mucilage. J Food Sci 54:1302-1305.

Mazza G and BD Oomah. 1995. Flaxseed, dietary fiber, and cyanogens. Chapter in SC Cunnane and LU Thompson, Eds., *Flaxseed in human nutrition*. Champaign IL: AOCS Press.

McCloy U, MA Ryan, PB Pencharz, RJ Ross, SC Cunnane. 2004. Comparison of the metabolism of eighteen-carbon ^{13}C-unsaturated fatty acids in healthy women. J Lipid Res 45:474-485.

Meagher LP, GR Beecher, VP Flanagan, BW Li. 1999. Isolation and characterization of the lignans, isolariciresinol and pinoresinol, in flaxseed meal. J Agric Food Chem 47:3173-3180.

Montgomery RD. 1980. Cyanogens. In *Toxic constituents of plant foodstuffs*, IE Liener, Ed. New York: Academic Press.

Morris D. 2003. *Flax—a health and nutrition primer*. Flax Council of Canada. Available online at http://www.flaxcouncil.ca/english/index.php?p=primer&mp=nutrition.

Mousavi Y and H Adlercreutz. 1992. Enterolactone and estradiol inhibit each others proliferative effect on MCF-7 breast cancer cells in culture. J Steroid Biochem Mol Biol 41:615-619.

Mueller RS, KV Fieseler, MJ Fettman, S Zabel, RA Rosychuk, GK Ogilvie, TL Greenwalt. 2004. Effect of omega-3 fatty acids on canine atopic dermatitis. JSmall Anim Pract 45:293-297.

Muir AD. 2006. Flax lignans—analytical methods and how they influence our understanding of biological activity. J AOAC Int 89:1147-1157.

National Toxicology Program. 1991. Salmonella study summary: linseed oil. Study no. 038199. Available at http://ntp-apps.niehs.nih.gov/ntp-tox

Nesbitt PD, Y Lam, LU Thompson. 1999. Human metabolism of mammalian lignan precursors in raw and processed flaxseed. Am J Clin Nutr 69:549-555.

Newcomer LM, IB King, KG Wicklund, JL Stanford. 2001. The association of fatty acids with prostate cancer risk. Prostate 47:262-268.

Niedzwiedz-Siegien I. 1998. Cyanogenic glucosides in *Linum usitatissimum*. Phytochem 49:59-63.

Niemeyer HB, DM Honig, SE Kulling, M Metzler. 2003. Studies on the metabolism of the plant lignans secoisolariciresinol and matairesinol. J Agric Food Chem 51:6317-6325.

Oomah BD. 2001. Flaxseed as a functional food source. J Sci Food Agric 81:889-894.

Oomah BD and G Mazza. 1993. Flaxseed proteins—a review. Food Chem 48:109-114.

Oomah BD and G Mazza. 1998. Compositional changes during commercial processing of flaxseed. Ind Crops Prod 9:29-37.

Oomah BD, G Mazza, E O Kenaschuk. 1992. Cyanogenic compounds in flaxseed. J Agric Food Chem 40:1346-1348.

Oomah BD, EO Kenaschuk, W Cui, G Mazza. 1995a. Variation in the composition of water-soluble polysaccharides in flaxseed. J Agric Food Chem 43:1484-1488.

Oomah BD, EO Kenaschuk, G Mazza. 1995b. Phenolic acids in flaxseed. J Agric Food Chem 43:2016-2019.

Oomah BD, EO Kenaschuk, G Mazza. 1995c. Safety considerations of flaxseed and flaxseed components. Flax Institute of the United States; 178-182.

Oomah BD, B Berekoff, ECY Li-Chan, G Mazza, EO Kenaschuk, SD Duguid. 2007. Cadmium-binding protein components of flaxseed: Influence of cultivar and location. Food Chem 100:318-325.

Orcheson LJ, SE Rickard, MM Seidl, LU Thompson. 1998. Flaxseed and its mammalian lignan precursor cause a lengthening or cessation of estrous cycling in rats. Cancer Lett 125:69-76.

Phipps WR, MC Martini, JW Lampe, JL Slavin, MS Kurzer. 1993. Effect of flax seed ingestion on the menstrual cycle. J Clin Endocrinol Metab 77:1215-1219.

Prasad K. 2000. Antioxidant activity of secoisolariciresino diglucoside-derived metabolites, secoisolariciresinol, enterodiol, and enterolactone. Int J Angiol 9:220-225.

Prasad K. 2005. Effect of chronic administration of lignan complex isolated from flaxseed on the hemopoietic system. Molec Cell Biochem 270:139-145.

Przybylski R and JK Daun. 2001. Additional data on the storage stability of milled flaxseed. JAOCS 78:105-106.

Radtke J, J Linseisen, G Wolfram. 1998. Phenolic acid intake of adults in a Bavarian subgroup of the national food consumption survey. Z Ernahrungswiss 37:190-197. [Article in German with English abstract]

Ramon JM, R Bou, S Romea, ME Alkiza, M Jacas, J Ribes, J Oromi. 2000. Dietary fat intake and prostate cancer risk: a case-control study in Spain. Cancer Causes Control 11:679-685.

Ratnayake WMN, WA Behrens, PWF Fischer, MR L'Abbe, R Mongeau, JL Beare-Rogers. 1992. Chemical and nutritional studies of flaxseed (variety Linott) in rats. J Nutr Biochem 3:232-240.

Reeves PG. 1997. Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr 127:838S-841S.

Rickard SE, LJ Orcheson, MM Seidl, L Luyengi, HHS Fong, LU Thompson. 1996. Dose-dependent production of mammalian lignans in rats and in vitro from the purified precursor secoisolariciresinol diglycoside in flaxseed. J Nutr 126:2012-2019.

Rickard SE and LU Thompson. 1998. Chronic exposure to secoisolariciresinal diglycoside alters lignan disposition in rats. J Nutr 128:615-623.

Rickard SE and LU Thompson. 2000. Urinary composition and postprandial blood changes in ^{3}H-secoisolariciresinol diglycoside (SDG) metabolites in rats do not differ between acute and chronic SDG treatments. J Nutr 130:2299-2305.

Rickard SE, YV Yuan, LU Thompson. 2000. Plasma insulin-like growth factor I levels in rats are reduced by dietary supplementation of flaxseed or its lignan secoisolariciresinol diglycoside. Cancer Lett 161:47-55.

Roberts A, BS Lynch, AP Wolterbeek, H Korte, M Unkila, DH Waalkens-Berendsen. 2006. Safety assessment of 7-hydroxymatairesinol HMR lignan. Paper presented at the 45th Annual Meeting of the Society of Toxicology, March, Sandiego; Abstract No. 2335.

Rojas-Molina M, J Campos-Sanchez, M Analla, A Munoz-Serrano, A Alonso-Moraga. 2004. Genotoxicity of vegetable cooking oils in the Drosophila wing spot test. Envir Molec Mutagen 45:90-95.

Rosling H. 1993. Cyanide exposure from linseed. Lancet341:177. [Letter to the Editor]

Sammour RH. 1999. Proteins of linseed (*Linum usitatissimum* L.), extraction and characterization by electrophoresis. Bot Bull Acad Sin 40:121-126.

Schaefer EJ, JL Augustin, MM Schaefer, H Rasmussen, JM Ordovas, GE Dallal, JT Dwyer. 2000. Lack of efficacy of a food-frequency questionnaire in assessing dietary macronutrient intakes in subjects consuming diets of known composition. Am J Clin Nutr 71:746-751.

Schilcher H and M Wilkens-Sauter. 1986. Quantitative bestmmung cyanogener glykoside in *Linum usitatissimum* mit hilfe der HPLC. Fette Seifen Anstrich 88:287-290.

Schottner M, D Gansser, G Spiteller. 1997. Interaction of lignans with human sex binding globulin (SHBG). Z Naturforsch Sect C J Biosci 52:834-843.

Schuurman AG, PA van den Brandt, E Dorant, HAM Brants, RA Goldbohm. 1999. Association of energy and fat intake with prostate carcinoma risk. Cancer 86:1019-1027.

Setchell KDR, AM Lawson, FL Mitchell, H Adlercreutz, DN Kirk, M Axelson. 1980. Lignans in man and in animal species. Nature 287:740-2.

Shahidi F, JK Daun, DR DeClercq. 1997. Chapter 9. Glucosinolates in brassica oilseeds-processing effects and extration. In *Antinutrients and Phytochemicals in Foods*; Shahidi, F (Ed); ACS: Washington.

Shultz TD, WR Bonorden, WR Seaman. 1991. Effect of short-term flaxseed consumption on lignan and sex hormone metabolism in men. Nutr Res 11:1089-1100.

000168

Sicilia T, HB Niemeyer, DM Honig, M Metzler. 2003. Identification and sterochemical characterization of lignans in flaxseed and pumpkin seeds. J Agric Food Chem 51:1181-1188.

Siemelink M. A Verhoef, A Opperhuizen, AH Piersma. 2001. Perinatal nutrition and postnatal development in the rat. Toxicologist 60:222. [Abstract]

Smeds AI, PC Eklund, RE Sjoholm, SM Willfor, S Nishibe, T Deyama, BR Holmbom. 2007. Quantification of a broad spectrum of lignans in cereals, oilseeds, and nuts. J Agric Food Chem 55:1337-1346.

Snyder DC, BR Switzer, V Hars, RT Vollmer, JF Madden, SL George, MT Ruffin, W Demark-Wahnefried. 2007. Flaxseed supplementation and/or dietary fat restriction alters fatty acid profiles in the diet, erythrocytes, and prostatic tissue in men with prostate cancer. J Am Diet Assoc 107(Suppl 1):A38. [Abstract]

Spence JD, T Thornton, AD Muir, ND Westcott. 2003. The effect of flax seed cultivars with differing content of α-linolenic acid and lignans on responses to mental stress. J Am Coll Nutr 22:494-501.

Sprando RL, TFX Collins, TN Black, N Olejnik, JI Rorie, M Scott, P Wiesenfeld, US Babu, M O'Donnell. 2000a. The effect of maternal exposure to flaxseed on spermatogenesis in F_1 generation rats. Food Chem Toxicol 38:325-334.

Sprando RL, TFX Collins, P Wiesenfeld, US Babu, C Rees, T Black, N Olejnik, J. Rorie. 2000b. Testing the potential of flaxseed to affect spermatogenesis: morphometry. Food Chem Toxicol 38:887-892.

Sprecher H. 2002. The roles of anabolic and catabolic reactions in the synthesis and recycling of polyunsaturated fatty acids. Prostaglandins, Leukot Essent Fatty Acids 67:79-83.

Stuglin C and K Prasad. 2005. Effect of flaxseed consumption on blood pressure, serum lipids, hemopoietic system and liver and kidney enzymes in healthy humans. J Cardiovasc Pharmacol Therapeut 10:23-27.

Tang AB, KY Nishimura, SD Phinney. 1993. Preferenctial reduction in adipose tissue alpha-linolenic acid (18:3 omega 3) during very low calorie dieting despite supplementation with 18:3 omega 3. Lipids 28:987-993.

Tarpila S, A Aro, I Salminen, A Tarpila, P Kleemola, J Akkila, H Adlercreutz. 2002. The effect of flaxseed supplementation in processed foods on serum fatty acids and enterolactone. Eur J Clin Nutr 56:157-165.

Tkachuk R. 1969. Nitrogen-to-protein conversion factors for cereals and oilseed meals. Cereal Chem 46 :419-423.

Thompson LU, JM Chen, T Li, K Strasser-Weippi, PE Goss. 2005. Dietary flaxseed alters tumor biological markers in postmenopausal breast cancer. Clin Cancer Res 11:3828-3835.

Tou JC and LU Thompson. 1997. The effect of flaxseed on reproductive development and function. FASEB J 11:A420. [Abstract]

Tou J, J Chen, LU Thompson. 1998a. The importance of timing of exposure to flaxseed on reproductive development of rats. FASEB J 12:A768. [Abstract]

Tou JCL, J Chen, LU Thompson. 1998b. Flaxseed and its lignan precursor, secoisolariciresinol diglycoside, affect pregnancy outcome and reproductive development in rats. J Nutr. 128:1861-1868.

Tou JCL, J Chen, LU Thompson. 1999. Dose, timing, and duration of flaxseed exposure affect reproductive indices and sex hormone levels in rats. J Toxicol Envir Health, Part A 56:555-570.

Tou JCL and LU Thompson. 1999. Exposure to flaxseed or its lignan component during different developmental stages influences rat mammary gland structures. Carcinogenesis 20:1831-1835.

U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS). 2000. *1994–96, 1998 Continuing Survey of Food Intakes by Individuals [CD-ROM],* data and documentation. Springfield, VA: National Technical Information Service. Accession No. PB2000-500027.

U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS). 2007. *National Nutrient Database for Standard Reference*, Release 20. Available at: *http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl.*

Vaisey-Genser M and DH Morris. 2003. Introduction—history of the cultivation and uses of flaxseed. In *Flax—the genus* Linum, AD Muir and ND Westcott, Eds. New York: Routledge.

Vanderveen JE. 1995. Regulation of flaxseed as a food ingredient in the United States. Chapter in SC Cunnane and LU Thompson, Eds., *Flaxseed in human nutrition.* Champaign IL: AOCS Press.

Velasquez MT, SJ Bhathena, T Ranich, AM Schwartz, DE Kardon, AA Ali, CC Haudenschild, CT Hansen. 2003. Dietary flaxseed meal reduces proteinuria and ameliorates nephropathy in an animal model of type II diabetes mellitus. Kidney Internat 64:2100-2107.

Vermunt SHF, RP Mensink, AMG Simonis, G Hornstra. 2000. Effects of dietary α-linolenic acid on the conversion and oxidation of [^{13}C] α-linolenic acid. Lipids 35:137-142.

000170

Wanasundara PKJPD, R Amarowicz, MT Kara, F Shahidi. 1993. Removal of cyanogenic glycosides of flaxseed meal. Food Chem 48:263-266.

Wanasundara PKJPD, UN Wanasundara, E Shahidi. 1999. Changes in flax (*Linum usitatissimum* L.) seed lipids during germination. J Am Oil Chem Soc 76:41-48.

Wang WQ, LQ Liu, CM Higuchi, HW Chen. 1998. Induction of NADPH:quinone reductase by dietary phytoestrogens in colonic Colo205 cells. Biochem Pharmacol 56:189-195.

Ward WE, J Chen, LU Thompson. 2001a. Exposure to flaxseed or its purified lignan during suckling only or continuously does not alter reproductive indices in male and female offspring. J Toxicol Envir Health, Part A 64:567-577.

Ward WE, YV Yuan, AM Cheung, LU Thompson. 2001b. Exposure to flaxseed and its purified lignan reduces bone strength in young but not older male rats. J Toxicol Envir Health, Part A 63:53-65.

Welshons WV, CS Murphy, R Koch, G Calaf, VC Jordan. 1987. Stimulation of breast cancer cells in vitro by the environmental estrogen enterolactone and the phytoestrogen equol. Breast Cancer Res Treat 10:169-175.

White WLB, DI Arias-Garzon, JM McMahon, RT Sayre. 1998. Cyanogenesis in cassava. Plant Physiol 116:1219-1225.

Wiesenfeld PW, US Babu, TFX Collins, R Sprando, MW O'Donnell, TJ Flynn, T Black, N Olejnik. 2003. Flaxseed increased α-linolenic and eicosapentaenoic acid and decreased arachidonic acid in serum and tissues of rat dams and offspring. Food Chem Toxicol 41:841-855.

Williams CM and G Burdge. 2006. Long-chain n-3 PUFA: plant v. marine sources. Proc Nutr Soc 65:42-50.

Wolterbeek AP, A Roberts, H Korte, M Unkila, DH Waalkens-Berendsen. 2004. Prenatal developmental toxicity study with 7-hydroxymatairesinol potassium acetate (HMR lignan) in rats. Regul Toxicol Pharmacol 40:1-8.

World Health Organization (WHO). 2007. *Cyanide in drinking water*. Geneva: WHO Press.

Yang H, Z Mao, H Tan. 2004. Determination and removal methods for cyanogenic glucoside in flaxseed. ASAE Annual International Meeting, Ottawa, Ontario. 6599-6604.

Yuan YV, SE Richard, LU Thompson. 1999. Short-term feeding of flaxseed or its lignan has minor influence on in vivo hepatic antioxidant status in young rats. Nutr Res 19:1233-1243.

Zöllner H and R Giebelmann. 2007. Cyanogenic glycosides in food: cultural historical remarks. Deutsche Lebensmittel-Rundschau 103:71-77. [Article in German with English abstract]

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CONCLUSION OF THE EXPERT PANEL: GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION FOR THE GRAS STATUS OF THE ADDITION OF WHOLE AND MILLED FLAXSEED TO CONVENTIONAL FOODS AND MEAT AND POULTRY PRODUCTS

Prepared for:
Flax Canada 2015 Inc.
Winnipeg, Canada

January 2009

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We, the members of the expert panel, have individually and collectively critically evaluated the publicly available information on whole and milled flaxseed summarized in a monograph prepared by JHEIMBACH LLC as well as other material deemed appropriate or necessary. Our evaluation included review of the identity and physical-chemical properties of whole and milled flaxseed, including their production methods and composition, the potential exposure resulting from the intended use of whole and milled flaxseed in conventional foods, and published research bearing on the safety of whole and milled flaxseed. Our summary and conclusion resulting from this critical evaluation are presented below.

Summary

- The product that is the subject of this GRAS determination is flaxseed, the seed of *Linum usitatissimum* L, which may be marketed and used as whole seed (measuring 3 to 5 mm in length) or milled to a powder varying in coarseness with particle diameters ranging from 200 to 1500 μm. Flaxseed is also known as linseed, although in America the term linseed is usually reserved for industrial products.

- Canada is the world's leader in production of flaxseed. In the U.S., the major production area is in North Dakota. Dozens of cultivars are currently grown and new ones are introduced every year; these cultivars vary slightly in their composition. The composition of flaxseed is also affected by growing area, weather, agronomic practices, harvesting time, and other factors.

- Dried flaxseed contains about 40-45% lipid, 30-35% carbohydrate, and 20-30% protein. The lipid content is generally 70 to 75% polyunsaturated fatty acids and about 15 to 20% oleic acid. About 80% of the polyunsaturated fatty acids are α-linolenic acid. Over 90% of the carbohydrate in flaxseed is in the form of non-digestible fiber, including both insoluble cellulose and soluble mucilage.

- Flaxseed also contains about 1.3% lignans. The primary lignan is 2,3-bis(3-methoxy-4-hydroxybenzyl)butane-1,4-diol, more often referred to by the trivial name secoisolariciresinol (SECO). SECO constitutes over 95% of the total lignan content of flaxseed, which also includes small amounts of matairesinol and trace amounts of pinoresinol and isolariciresinol. The lignans rarely exist in aglyconic form; SECO invariably occurs in the form of a diglucoside refered to as secoisolariciresinol diglucoside, or SDG. After ingestion, about 90% of the flaxseed lignans SECO and matairesinol are converted by colonic microbiota into the mammalian lignans enterodiol and enterolactone.

- Like many other edible plants, flaxseed contains the cyanogenic glycosides linustatin, neolinustatin, linamarin, and lautostralin, which release hydrogen cyanide on hydrolysis. These compounds, which are synthesized from amino acids, are present at variable concentrations ranging from about 0.4 to 0.9%. When flaxseed is crushed in water, action of the enzyme β-glycosidase releases hydrogen cyanide, which is then washed away.

- Whole and milled flaxseed are intended to be added to conventional foods, limited only by cGMP, to provide both α-linolenic acid and dietary fiber in order to increase the intake of these nutritional components. It is anticipated that the average intake of flax from one serving of a flaxseed-containing food will not exceed 4 g, and that daily consumption will rarely exceed four servings of foods that provide flaxseed. Thus, the estimated daily intake of flaxseed from its intended use is 16 g/day or less.

- Flaxseed has been consumed for millennia, and is widely added to foods—particularly bakery products—in Canada. Flaxseed is also added to a number of currently marketed foods in the U.S. and the Food and Drug Administration has repeatedly stated that it perceives no hazard from addition of flaxseed at up to 12% in foods. Most recently, the FDA accepted the safety of human consumption of a genetically modified flax strain based on its substantial compositional equivalence with its parent strain.

- A large body of published toxicological data exists to demonstrate the safety of the intended use of flaxseed. This includes three subchronic oral toxicity studies in rats and one in piglets, as well as two subchronic oral toxicity studies of SDG in rats and one in rabbits. No adverse effects were observed in any of these studies, which included maximum doses of 30,000 mg/kg bw/day for flaxseed and 63 mg/kg bw/day for SDG.

- Additionally, nine developmental and reproductive toxicity studies have been conducted on flaxseed and five on α-linolenic acid or SDG, with flaxseed doses ranging from 3,750 to 30,000 mg/kg bw/day. While weak estrogenic effects were observed at high dose levels, with great variability across studies, no consistent effects were noted at lower doses.

- More than thirty studies in which flaxseed was administered to humans found a variety of beneficial effects such as improved laxation and improvement in blood lipid profiles, but no indication of intolerance or adverse effect at any tested dose level.

- While several cohort and case-control studies have suggested a possible link between high intakes of α-linolenic acid, found in relatively high concentration in flaxseed, and prostate cancer, methodological weaknesses of these studies limit their persuasiveness. No linkage between flaxseed or α-linolenic acid intake and prostate cancer has been observed in any of the several controlled studies investigating this issue. Further, the amount of α-linolenic acid provided by the intended use of whole and milled flaxseed is well within recommended levels and well below those levels putatively implicated in prostate cancer.

Conclusion

We, the undersigned expert panel members, are qualified by scientific education and experience to evaluate the safety of ingredients intended to be added to foods. We have individually and collectively critically evaluated the materials summarized above.

We observe that flaxseed products have a long history of safe use in foods in the U.S. and internationally and are appropriately regarded as safe and nutritious. We note that extensive animal and human research has confirmed the safety of flaxseed and its constituents. We find that the composition and purity of whole and milled flaxseed have been adequately characterized to assure a wholesome food-grade product. Therefore, we conclude that addition of whole and milled flaxseed to conventional foods limited only by cGMP is safe.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusion. Therefore, the intended use of whole and milled flaxseed is safe, and is GRAS, via scientific procedures.

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