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CANOLA COUNCIL
OF CANADA

1999 OCT 22 P 1:27

October 8, 1999

400-167 Lombard Ave.
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Ms. Paulette Gaynor
Regulatory Policy Branch, HFS-200
Office of Premarket Approval
Center for Food Safety & Applied Nutrition
U.S. Food & Drug Administration
200 C Street S.W.
Washington, D.C.
20204

Dear Ms. Gaynor:

Re: Food Approval of Low Erucic Acid Rapeseed Oil (Canola Oil) Derived from Canola-Quality *B. juncea*

In response to our telephone conversation earlier this week and the material you kindly faxed to me, I am responding with the attached:

1. A revised Statement of Claim which, I trust, you will find adequately addresses the required format outlined in the procedures for GRAS notification; and
2. Copies of the notification document provided to you in triplicate as required by the GRAS notification procedures.

As outlined in my earlier notification dated September 17, 1999:

- Canola-quality *B. juncea* was developed through traditional plant breeding methods by a collaborative breeding program of Agriculture & Agri-Food Canada and Saskatchewan Wheat Pool.
- Canola-quality *B. juncea* is substantially equivalent in composition and nutritional value compared with traditional canola oil and meets the specifications listed for canola oil.
- The 23rd Session of the Codex Alimentarius Commission (Rome, 28 June - 3 July 1999) adopted *B. juncea* as a source of low erucic acid rapeseed oil in their revised Standard for Named Vegetable Oils (Section 2.1.13 of Appendix II). The Codex Alimentarious Commission also recognized canola as a common name (or synonym) for low erucic acid rapeseed.
- It is the canola industry's intention, after all regulatory requirements are met, to commercialize canola quality *juncea* as canola without segregation from the present *napus* and *rapa* canola production.

If you have any questions or comments, please do not hesitate to contact me. Thank you for your consideration in this matter.

Sincerely,

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Dale Adolphe
President

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LOW ERUCIC ACID RAPESEED OIL†

derived from canola-quality (low erucic acid, low glucosinolate)

Brassica juncea (L.) Czern.

A GRAS Notification submitted by the

Canola Council of Canada, Winnipeg, Manitoba

to the

United States Food and Drug Administration, Washington, D.C.

in accordance with proposed Section 170.36 of the

Federal Food, Drug, and Cosmetic Act

September, 1999

† The 23rd Session of the Codex Alimentarius Commission (Rome, 28 June - 3 July 1999) has adopted a revised Standard for Named Vegetable Oils. Section 2.1.13 of Appendix II of the Standard includes *Brassica juncea* as a source of low erucic acid rapeseed oil.

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1. STATEMENT OF CLAIM

The Canola Council of Canada claims that the use of refined, low erucic acid rapeseed oil derived from seed of canola-quality (low erucic acid, low glucosinolate) *Brassica juncea* in food processing, in the manufacture of margarines and shortenings, and as a salad and frying oil, is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because it (the Canola Council of Canada) has been determined that such use is generally recognized as safe (GRAS).

This GRAS determination is based on scientific data that indicates substantial equivalency of oil derived from canola-quality *B. juncea* to canola oil (low erucic acid rapeseed oil), which was declared GRAS by the Food and Drug Administration (FDA) in 1985. Oil derived from canola-quality *B. juncea* will be indistinguishable from canola oil derived from *B. napus* or *B. rapa* (formerly *B. campestris*) with respect to the specifications listed for canola oil in the Food Chemicals Codex, 4th Edition (1996), and will be sold and utilized as canola oil.

The scientific data and information that are the basis for this GRAS determination are available for the Food and Drug Administration's review. These will be supplied to the FDA upon request to the claimant's address outlined below.

October 8, 1999
Date

Dale F. Adolphe, President
Canola Council of Canada
400-167 Lombard Avenue
Winnipeg, Manitoba
Canada R3B 0T6

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2. IDENTITY OF THE NOTIFIED SUBSTANCE

2.1 Formal name: Low erucic acid rapeseed oil (LEAR oil) derived from canola-quality *Brassica juncea*.

2.2 Common or usual name: Low erucic acid rapeseed oil derived from canola quality *Brassica juncea* will be marketed as canola oil.

2.3 Chemical Abstracts Service Registry Number: The number (8002-13-9) assigned to rapeseed oil, and used for canola oil, would be applicable.

2.4 Development of canola-quality (low erucic acid, low glucosinolate) *Brassica juncea*

2.4.1 Background

Canola (*Brassica napus* and *B. rapa*) is an edible oil crop developed during the 1970's by genetic alteration of rapeseed. It is grown widely in Canada, several European countries, and Australia, and to a lesser extent in the United States. Canola is distinguished from traditional rapeseed by its greatly reduced levels of erucic acid (C22:1, 13-*cis*) and glucosinolates. Whereas traditional rapeseed oil contains 25-45% of erucic acid, and rapeseed meal, 100-150 µmoles of aliphatic glucosinolates per gram of air-dry, oil-free solids, canola oil and meal contain less than 2% of erucic acid and less than 30 µmoles of aliphatic glucosinolates per gram, respectively. These alterations have led to the widespread use of canola oil in Canada, and elsewhere, as a salad and frying oil and in the manufacture of margarines, shortenings, and other food products. The meal by-product is used as a high-protein feed ingredient in rations for cattle, swine and poultry.

Brassica juncea is higher yielding, more heat and drought tolerant, and more resistant to blackleg (a fungal disease) than *B. napus* and *B. rapa* and earlier maturing and less prone to seed loss due to shattering than *B. napus*. These attributes make *B. juncea* well adapted to the semi-arid growing conditions of the Canadian prairies and US Great Plains. It is anticipated that the development of canola-quality (low erucic acid, low glucosinolate) *B. juncea* will enable reliable production of canola on several hundred thousand additional hectares of land in southeastern Alberta and southwestern Saskatchewan, without irrigation. Development of canola-quality *B. juncea* also has the potential to expand canola acreage in the U.S., particularly in the great plains region of the west and midwest.

Traditional plant breeding methods were used to develop canola-quality *B. juncea*. Changes in fatty acid composition were brought about by isolation of genetic variants, crossing, and selection within the *B. juncea* species. The low glucosinolate characteristic was incorporated from the canola species *B. rapa* by means of an interspecific cross. Low erucic acid rapeseed (LEAR) oil from commercial, licensed varieties of canola-quality *B. juncea* will be substantially equivalent in its fatty acid composition and physiochemical properties to canola oil derived from *B. napus* and *B. rapa*, and will be suitable for use in food processing, in the manufacture of margarines and shortenings, and as a salad and frying oil.

2.4.2 Development protocol

Plant breeders have been able to develop better quality varieties for a number of different oilseeds by the traditional method of selection amongst existing variation within a species Genotypes of *B. napus* (Downey and Harvey, 1963) and *B. rapa* (Jonsson, 1973) were

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developed by screening a wide range of germplasm for fatty acid and glucosinolate composition, and then selecting material that best met the required quality parameters. In this way, germplasm exhibiting low levels of erucic acid and 2-propenyl (allyl) and butenyl aliphatic glucosinolates was selected, paving the way for the development of canola. Conventional selection techniques were similarly utilized in the development of canola-quality *B. juncea.*

A detailed description of *B. juncea* and its relationship to *B. napus*, *B. rapa* and other *Brassica* species was first published by U (1935) (Figure 1). *B. rapa* was previously referred to as *B. campestris*, but taxonomists have now changed the species name to reflect its original designation. *B. juncea* is a digenomic (*ab*), amphidiploid species that contains the genomes of the two monogenomic diploid species, *B. rapa* (*a*-genome) and *B. nigra* (*b*-genome). Gland *et al.* (1981) reported that *B. rapa* synthesizes 3-butenyl and 4-pentenyl glucosinolates and, rarely, 2-propenyl (allyl) glucosinolates. *B. nigra* synthesizes only 2-propenyl glucosinolates. As an amphidiploid, *B. juncea* can accumulate glucosinolates that are present in either or both of its parent species. *B. juncea* germplasm is usually divided into two main groups. The Indian type contains 40-45% of erucic acid and a mixture of allyl and butenyl aliphatic glucosinolates. It is characterized by a brown seed coat and day-length insensitivity. The European/Chinese type generally contains 25-35% of erucic acid and allyl glucosinolates only. It can have either a clear or a brown seed coat, and is day-length sensitive (Hemingway, 1995). The *B. juncea* condiment mustards grown in Canada are of the European/Chinese type.

Canola-quality *Brassica juncea* development programs have been established in several countries, including the U.S., Australia, Sweden, Pakistan and India, as well as Canada. The cooperative breeding program of the Saskatoon Research Centre of Agriculture and Agri-Food Canada (AAFC) and the Saskatchewan Wheat Pool appears to be the most advanced. Zero erucic acid germplasm was first identified in two European/Chinese type *B juncea* lines in Australia in the early 1980's. Researchers at the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Canberra reported the selection of the Zem1 and Zem2 lines which contained essentially no erucic acid (Kirk and Oram, 1981; Kirk and Hurlstone, 1983). Agriculture and Agri-Food Canada initiated a breeding program in Saskatoon to develop *B. juncea* canola in 1985. The program at AAFC has focused on the development of *B. juncea* with levels of allyl glucosinolates reduced to as near zero as possible, and levels of other glucosinolates similar to those in *B. napus* and *B. rapa* canola

Low glucosinolate *B. juncea* was developed at AAFC through an interspecific cross between an Indian *B. juncea* line containing only 3-butenyl-type glucosinolates and a low-glucosinolate, zero erucic acid *B. rapa* line. The original interspecific F_1 generation was then backcrossed to Indian *B. juncea* (Love, 1988; Love *et al.*, 1990). This research led to the development of the low glucosinolate *B. juncea* line, 1058. Field-grown progeny of this line contained less than 10 µmoles of total glucosinolates per gram of meal but had very low fertility and oil content, and high erucic acid levels. In order to combine the low glucosinolate characteristic with zero erucic acid, and to increase oil content, progeny from line 1058 were crossed with line LDZ. LDZ was developed at AAFC through a cross between a Russian high oil mustard line (Donskaja) and Zem 1, followed by a backcross to Donskaja (Woods and Rakow, 1987). LDZ is a high oil line with zero erucic acid. The F_1 progeny of this cross was then crossed with the Canadian *B. juncea* Oriental mustard variety, Cutlass, to produce adapted *B. juncea* germplasm that combined genes for zero erucic acid and high oil content,

low glucosinolate levels, high yield, early maturity and resistance to white rust disease (Love *et al.*, 1991). Pedigree selection in segregating generations derived from these crosses resulted in the identification of well adapted, high yielding, blackleg resistant, canola-quality *B. juncea* lines (Rakow *et al.*, 1995). The pedigree of these early canola-quality *B. juncea* lines is illustrated in Figure 2.

Two breeding populations (P and R) which differed in oil and glucosinolate contents were developed from the material described by Rakow *et al.*, (1995). F_4 lines were field tested in 1990. The aliphatic glucosinolate contents in lines of both populations were less than 30 µmoles per gram of meal, the maximum concentration of glucosinolates allowed for a canola cultivar. Research priorities for this material included reducing the level of allyl glucosinolates to as near zero as possible, and total glucosinolates to 15 to 20 µmoles per gram of meal. In addition, the fatty acid profiles of these lines differed slightly from that of canola in that they were lower in oleic acid (45.6%, in comparison to approximately 60% in canola) and higher in linoleic acid (31.8%, in comparison to approximately 22% in canola) and in linolenic acid (13.0%, in comparison to approximately 11% in canola).

Lines from the P and R populations developed at AAFC were used by the Saskatchewan Wheat Pool as the initial germplasm for their *B. juncea* canola breeding program. The objectives of this research program were to continue to reduce allyl glucosinolate concentrations to as near zero as possible and to obtain a fatty acid profile as similar to that of canola as possible. From a cross between P and R lines, two plants were selected that had fatty acid profiles similar to that of *B. napus*. In 1995, crosses were made between the progeny of these plants and other *B. juncea* lines containing less than one µmole of allyl glucosinolate per gram of whole seed. Microspore-derived doubled haploid (DH) plants were produced from these crosses. Progeny from these DH plants were assessed in nursery rows in 1996 and in multi-location trials in 1997. In 1998, seed increase plots of two of these lines were grown in Saskatchewan. Seed of one line, PC97-03, which was of canola-quality with respect to both glucosinolates (Table 1) and erucic acid (Table 2), was used in a test crush to determine the chemical and physical characteristics of the refined oil. The pedigree of PC97-03 is illustrated in Figure 3. The Canola Council of Canada (1994) definition of canola is presented in section 2.8.

2.5 Chemical composition of low erucic rapeseed (LEAR) oil derived from canola-quality *B. juncea*

Low erucic acid rapeseed oil is derived from canola-quality *B. juncea* seed by the same extraction and refining processes that are used for *B. napus* and *B. rapa* canola seed, typically prepress-solvent extraction, followed by degumming, alkali or physical refining, bleaching and deodorization. Like other vegetable oils, LEAR oil derived from canola-quality *Brassica juncea* is a mixture of triglycerides that differ in the nature and distribution of their constituent fatty acids. The 1-2% of non-triglyceride lipid components present in the oil (the unsaponifiable fraction) includes sterols, tocopherols, free fatty acids, pigments and other minor constituents.

2.5.1 Fatty Acids

The fatty acid profile of LEAR oil derived from canola-quality *B. juncea* is similar to that of canola oil derived from *B. napus* and *B. rapa* (Table 2). Like canola, LEAR oil derived from canola-quality *B. juncea* contains five principal fatty acids - palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids. Lesser amounts of four other

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long-chain fatty acids (C20:0, C20:1, C22:1, and C24:1) are also present in LEAR oil derived from canola-quality *B. juncea* at levels comparable to those found in canola oil. The 0.2% level of erucic acid (C22:1) easily meets the canola requirement of not more than 2.0%. Line J90-4316 (column 2 of Table 2) is an early generation, low erucic *B. juncea* line which yields oil low in erucic acid and other long chain fatty acids, but higher in linoleic acid and lower in oleic acid than canola oil (column 4 of Table 2). Oil from a later generation canola-quality *B. juncea* line (PC97-03, column 3 of Table 2) possesses a fatty acid profile which is virtually indistinguishable from that of canola oil. Commercial varieties of canola-quality *B. juncea* will possess the fatty acid profile of this later generation line. For comparative purposes, the fatty acid profile of oil from *B. juncea* (Oriental) mustard has been included (column 1 of Table 2). Differences between its fatty acid profile, particularly the levels of erucic acid, eicosenoic acid (C20:1) and oleic acid, and those of canola oil and LEAR oil derived from canola-quality *B. juncea*, are evident.

2.5.2 Unsaponifiable Fraction

The unsaponifiable fraction of refined canola oil generally represents 1.0 to 1.5% of the oil by weight and consists of various sterols, tocopherols, pigments, free fatty acids and other minor constituents (Ackman, 1983; Przybylski, 1994). Boguski *et al.* (1998) determined that pilot-scale-extracted and refined LEAR oil derived from canola-quality *B. juncea* contained 0.99% of total unsaponifiable compounds.

2.5.3 Sterols

Sterols are present in canola oil as either free sterols or esterified sterols, with the quantities of each being similar (Ackman, 1983; Przybylski, 1994). Sterols contribute little to the functional properties of vegetable oils, but are recoverable from deodorizer distillate as valuable by-products. During refining, a portion of the sterols is also removed with the soapstock (Ackman, 1983; Przybylski, 1994).

The concentrations of the three major sterols in LEAR oil derived from canola-quality *B. juncea* and in canola oil are presented in Table 3. Campesterol and ß-sitosterol were the predominant sterols in both oils, and both contained brassicasterol, a C28 sterol that is characteristically found in oils derived from members of the genus *Brassica*. It is not found in significant quantities in other common edible vegetable oils and, therefore, can be used as a means of identifying *Brassica* oils that are present either alone or blended with other oils (Ackman, 1983). The concentrations of the three sterols in crude LEAR oil derived from canola-quality *B. juncea* (column 1 of Table 3) were similar to those in crude canola oil (columns 4 and 5 of Table 3). The sterol concentrations were reduced in both refined LEAR oil derived from canola-quality *B. juncea* (column 2 of Table 3) and refined canola oil (column 6 of Table 3) relative to the respective crude oils, although the ratios of the concentrations were relatively unaffected.

2.5.4 Tocopherols

Tocopherols are naturally-occurring antioxidants, and higher levels are associated with more highly unsaturated oils. Tocopherols have nutritional significance as sources of Vitamin E activity. As was the case with sterols, tocopherols are removed, in part, during refining. Gamma tocopherol and alpha tocopherol are predominant in canola oil (Ackman, 1990; Przybylski, 1994).

Gamma tocopherol was predominant in both crude LEAR oil derived from canola-quality *B. juncea* (column 1 of Table 4) and in crude canola oil (columns 4 and 5 of Table 4); similar concentrations of gamma tocopherol were detected in the two crude oils. The concentration of delta tocopherol was higher, and the concentration of alpha tocopherol lower, in crude LEAR oil derived from canola-quality *B. juncea* than in crude canola oil. Refining reduced the concentration of delta tocopherol, in particular, in both oils, whereas the concentrations of gamma tocopherol and alpha tocopherol were relatively unaffected (columns 2 and 6 of Table 4).

2.6 Allergens in *Brassica* species

Allergens associated with *Brassica* species fall into three categories, namely pollen-derived allergens (both proteinaceous and volatile), cutaneous allergens (presumably isothiocyanates), and seed-borne proteinaceous allergens (Dannaker and White, 1987; Focke *et al.,* 1998; Singh *et al.,* 1995).

The literature contains reports on the identification and characterization of allergenic proteins (those capable of recognizing human IgE from mustard-sensitive individuals or otherwise implicated in allergic responses) in seed of *Brassica* species. Most studies have involved white mustard (*Sinapis alba*), although Oriental mustard (*Brassica juncea*) and rapeseed (*Brassica napus*) have also been examined (Dominguez *et al.,* 1990; Gonzalez de la Pena *et al.,* 1996; Monsalve *et al.,* 1993, 1997). In some instances, the botanical identity of the material studied is uncertain. The most widely studied mustard allergen is a 2S seed storage albumin in *Sinapis alba*, the so-called Sin a I allergen, which appears to exist in multiple isoforms (Gonzalez de la Pena *et al.,* 1993, 1996; Menendez Arias *et al.,* 1988, 1990; Onaderra *et al.*, 1994). Similar 2S albumins have been identified in *B. juncea*, *B. napus*, and several other plant species (Bartolome *et al.,* 1997; Gonzalez de la Pena *et al.,* 1991; Monsalve *et al.,* 1991a, 1991b, 1993, 1997; Teuber *et al.,* 1998;). The 2S allergen from Oriental mustard (*B. juncea*) has been well characterized and is closely related to the Sin a I allergen from *S. alba* (Gonzalez de la Pena *et al.,* 1991; Monsalve *et al.,* 1993).

In light of its ancestry and developmental protocol, it is anticipated that seed of canola-quality *Brassica juncea* will bear the allergenic 2S storage proteins, and any other allergens, characteristic of rapeseed and mustard. There is no expectation that any new allergens have been introduced. The seed-borne proteinaceous allergens would arguably be of most potential relevance to the derivation of edible oil from canola-quality *B. juncea* seed. A recent review on the allergenicity of edible oils (Hefle and Taylor, 1999) states that "refined oils are safe for the food-allergic population to consume". Since refined oil will be derived from canola-quality *B. juncea* via processes identical to those now applied to canola (*i.e.*, pre-press solvent extraction, degumming, alkali or physical refining, bleaching and deodorization), there is no reason to believe that seed borne allergens will be a concern in refined oil derived from canola-quality *B. juncea*.

2.7 Accumulation of heavy metals in *Brassica* species

It is well known that *Brassica* species are capable of concentrating heavy metals in their vegetative tissues, to the extent that mustard, for example, has been investigated as a component of, and incorporated into, land reclamation strategies (Banuelos *et al.,* 1997, Begonia *et al.,* 1998; Kumar *et al.,* 1995; Salt *et al.,* 1995; Schafer *et al.,* 1998). This accumulation phenomenon is not unique to *Brassica* species, but rather is characteristic of plants that synthesize metal-binding proteins, the so-called phytochelatins (Salt *et al.,* 1995).

Of possible concern with respect to edible oil derived from canola-quality *B. juncea* would be the concentrations of heavy metals in seed tissue, and the extent to which heavy metals are transferred to the oil phase during oil extraction. Refined oil derived from canola-quality *B. juncea* line PC97-03 contained less than 1.53 mg/kg of heavy metals and less than 0.1 mg/kg of lead (Table 5). Food Chemicals Codex (1996) specifications for canola oil are less than or equal to 5 mg/kg for heavy metals and less than 0.1 mg/kg for lead.

Agriculture and Agri-Food Canada recently conducted a study (unpublished) on cadmium concentrations in *Brassica napus*, *B. rapa*, *B. juncea*, and canola-quality *B. juncea* at several locations in Saskatchewan (Selles and James, 1997). All of the *Brassica* species were high cadmium accumulators. Seed cadmium concentration was a function of both environment and cultivar, and no relationship existed between the cadmium concentration in seed and that in straw. Seed cadmium concentrations were elevated and highly variable at high cadmium sites, where the concentration of cadmium in seed of canola-quality *B. juncea* tended to be similar to that in *B. napus* seed, and higher than that in *B. rapa* seed and in *B. juncea* (Oriental mustard) seed. At low cadmium sites, seed of canola-quality *B. juncea* and that of *B. napus* contained similar concentrations of cadmium, and both were lower in cadmium than either *B. rapa* or mustard seed. Refined oil derived from canola-quality *B. juncea* line PC97-03 contained less than 0.03 mg/kg of cadmium, the detection limit for the assay employed (Boguski *et al.,* 1998).

2.8 Specifications for low erucic acid rapeseed oil derived from *B. juncea*

Low erucic rapeseed oil will be derived from seed of the species *Brassica juncea* which, compositionally, fit the Canola Council of Canada (1994) definition of canola: "seed of the species *Brassica napus* or *Brassica rapa*, the oil component of which seed contains less than 2% of erucic acid and the solid component of which seed contains less than 30 µmoles of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy, 3-butenyl glucosinolate, and 2-hydroxy, 4-pentenyl glucosinolate (aliphatic glucosinolates) per gram of air dry, oil free solid", and which is also essentially devoid of 2-propenyl (allyl) glucosinolate. In addition, oil from commercial varieties of canola-quality *B. juncea* will meet all of the specifications for canola oil as listed in the Food Chemicals Codex, 4th Edition (1996) (Table 5).

2.9 Characteristic properties and utilization of low erucic acid rapeseed (LEAR) oil derived from *Brassica. juncea*

Due to its substantial equivalence to canola oil with respect to composition (Table 5), it is anticipated that LEAR oil derived from canola-quality *B. juncea* will be marketed and utilized as canola oil, principally as a salad and cooking oil and in the manufacture of margarines and shortenings and other food products. The significant concentration of linolenic acid in LEAR oil derived from canola-quality *B. juncea* will negatively affect its oxidative stability, unless hydrogenated, as is the case for canola oil and soybean oil.

Canola oil is the most popular all-purpose vegetable oil in Canada, and is the preferred oil for use in both the U.S. and Canada where labeling regulations allow a claim of "low in saturated fats". During the period January-May, 1996, canola oil accounted for 78% of total Canadian production of deodorized edible oils (Statistics Canada, 1996). In terms of domestic production of food products, canola oil accounted for 88% of salad and cooking oils, 71% of shortening oils, and 53% of margarine oils. Canola oil is used for similar purposes in the United States, where it represents 7% of total vegetable oil use (USDA,

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1995). This equates to total annual *per capita* consumption in the U.S. of 1.7 kg as margarine, 0.3 kg as shortening, 0.8 kg as salad oil, and 0.8 kg as cooking oil. Complete substitution of LEAR oil derived from canola-quality *B. juncea* for canola oil is unlikely, as canola-quality *B. juncea* will represent only a portion of total Canadian and U.S. canola production.

3. BASIS FOR THE GRAS DETERMINATION

The claim for exemption of LEAR oil derived from canola-quality *B. juncea* from premarket approval is based on: 1) scientific procedures, specifically the substantial equivalence in composition of LEAR oil derived from canola-quality *B. juncea* to that of canola oil, and its derivation from seed of a *Brassica* species which has been bred to be low in glucosinolates and essentially free of allyl glucosinolates; and 2) the GRAS status and history of use of canola oil in the U.S.

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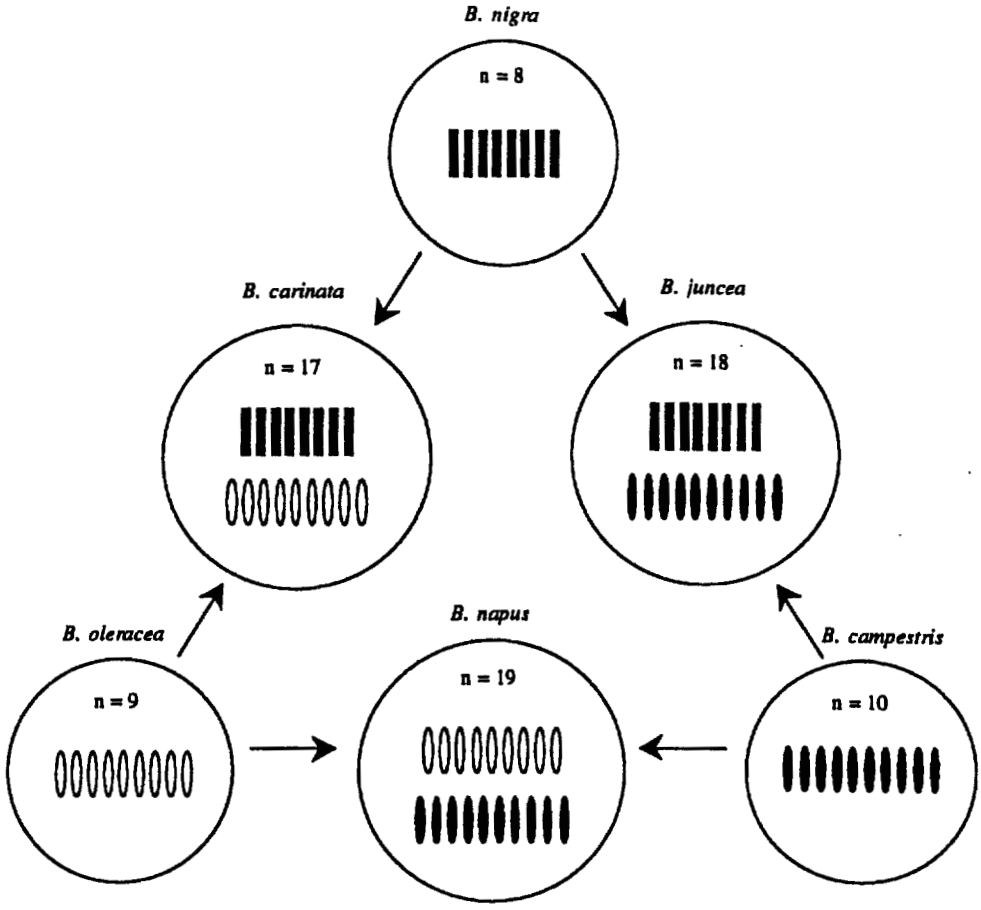

Figure 1: The triangle of U. The basic diploid species have chromosome numbers of 8, 9, and 10. These species have combined as shown to form a series of amphidiploids. Gametic chromosome (n) numbers are given.

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Figure 2: Pedigree of early breeding lines of canola-quality *B. juncea* developed at AAFC, Saskatoon.[1]

Year	Pedigree	Objective
1985	1058 X LDZ	• Increase oil content and introduce zero erucic acid; increase yield; improve maturity and introduce white rust resistance.
	↓	
1986	Cutlass X F_1	
	↓	
	F_1	• Select heterozygous lines for zero erucic acid and glucosinolate levels.
1987	↓	
	F_4	
	↓	
1988	F_4 X LDZ	• Increase oil content.
	↓	
	F_1	• Produce F_2 generation.
	↓	
	F_2	• Select for low glucosinolate levels.
1989		
	↓	
	F_3	• Select for zero erucic acid and white rust resistance.
	↓	
1990	F_4	• Select for agronomic type, high oil content, and low glucosinolate levels.

[1] Adapted from Love *et al.*, 1991.

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Figure 3: Pedigree of increased oleic acid and zero allyl glucosinolate breeding lines of canola-quality *B. juncea* developed at Saskatchewan Wheat Pool, Saskatoon.

Year	Pedigree
1992	J90-3450 X J90-4316
	↓
1993	F_2 Bulk Population
	↓
1994	F_3 Bulk*
	↓
1995	F_4 Single plants X 0 allyl DH Lines
	↓
1995-96	DH Plants (Eg. J96D-0758 = PC97-03)
	↓
1996	DH Nursery
	↓
1997	Yield trial
	↓
1998	Seed increase and test crush line PC97-03

*Plants with increased oleic acid levels were identified.

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Table 1. Glucosinolate profiles (µmoles per gram of air dry, oil free meal) of *B. juncea* cv. Cutlass (Oriental mustard), J90-4316 (low erucic acid, low glucosinolate *B. juncea*), PC97-03 (canola-quality *B. juncea*) and canola.

Glucosinolates (µmoles/g meal)	*B. juncea*[1] (cv. Cutlass)	*B. juncea*[2] (line J90-4316)	*B. juncea*[3] (line PC97-03)	*B. napus/B. rapa*[4] (typical crop values)
Allyl	162 5	3 1	0.4	0 2
Butenyl	1 5	14 4	7.1	1.2
Pentenyl	0.2	0 7	0.4	0 4
OH-Butenyl	-	0 9	1 0	7.6
OH-Pentenyl	0.3	-	-	0.5
OH-Indolyl	3.1	6 6	2 5	6 5
Total	168.5	25 7	11 4	16.4

[1] Data from Saskatchewan Wheat Pool
[2] J90-4316 is an early generation, low erucic acid *B. juncea* line which yields oil low in erucic acid and other long chain fatty acids, but which is higher in linoleic acid and lower in oleic acid than canola oil
[3] Boguski *et al* , 1998
[4] Daun and Adolphe, 1997

Table 2. Fatty acid composition (% of total fatty acids) of *B. juncea* cv. Cutlass (Oriental mustard), J90-4316 (low erucic acid, low glucosinolate *B. juncea*), PC97-03 (canola-quality *B. juncea*) and the canola crop average for western Canada, 1988-1997.

Fatty Acid	*B. juncea*[1] (cv. Cutlass)	*B. juncea*[2] (line J90-4316)	*B. juncea*[3] (line PC97-03)	*B. napus/B. rapa*[4] (1988-97 average)
C16:0	3 3	4.2	3.9	3 7
C18:0	1 2	1.9	2.6	1 7
C18:1	17 2	45 2	62 7	59 8
C18:2	21 4	31 8	17 2	20 4
C18:3	14 1	13.3	9 5	10.7
C20:0	0.7	0 6	0 7	0 6
C20:1	11 4	1 2	1 6	1.5
C22:1	25 8	0 1	0.2	0.5
C24:1	1 7	0 5	0.3	0 2

[1] Downey and Rimmer, 1993.
[2] Kruger and Mikle, 1993.
[3] Boguski *et al.*, 1998.
[4] DeClercq, 1998.

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Table 3. Sterol concentrations in oil derived from canola-quality *B. juncea*, mustard and canola.

Sterol	*B. juncea*[1] (line PC97-03) LEAR (Crude)	*B. juncea*[1] (line PC97-03) LEAR (Refined)	*B. juncea*[2] (cv. Cutlass) Mustard (Crude)	*B. napus*[2] (cv. Excel) Canola (Crude)	*B. rapa*[2] (cv. Parkland) Canola (Crude)	Commercial Canola (Refined)[3]
			CONCENTRATIONS (mg/kg)			
Brassicasterol	1342	634	1604	1146	1277	391
Campesterol	3333	2218	2585	3274	3556	1471
ß-sitosterol	6527	4037	5077	4779	5334	2336
			Ratios			
ß-sitoserol: Campesterol	2.0	1 8	2 0	1.5	1 5	1.6
ß-sitosterol: Brassicasterol	4 9	6.4	3.2	4 2	4.2	4 2
Campesterol: Brassicasterol	2 5	3 5	1 6	2.9	2 8	2 8

[1] Boguski *et al* , 1998
[2] Data from Saskatchewan Wheat Pool
[3] Data from CanAmera Foods.

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Table 4. Tocopherol concentrations in oil derived from canola-quality *B. juncea*, mustard, and canola.

Sterol	*B. juncea*[1] (line PC97-03) LEAR (Crude)	*B. juncea*[1] (line PC97-03) LEAR (Refined)	*B. juncea*[2] (cv. Cutlass) Mustard (Crude)	*B. napus*[2] (cv. Excel) Canola (Crude)	*B. rapa*[2] (cv. Parkland) Canola (Crude)	Commercial[3] Canola (Refined)
			CONCENTRATIONS (mg/kg)			
Delta	93	TR[4]	13	62	36	26
Gamma	414	297	395	490	605	526
Alpha	89	81	138	264	301	243
			Ratios			
Gamma: Alpha	4 7	3 7	2 9	1 9	2 0	2 2
Gamma: Delta	4 5	--	30.4	7 9	16 8	20 2
Alpha: Delta	1 0	--	10.6	4.3	8 4	9.4

[1] Boguski *et al.*, 1998.
[2] Data from Saskatchewan Wheat Pool.
[3] Data from CanAmera Foods
[4] Trace

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Table 5. Food Chemicals Codex (1996) specifications for canola oil and data obtained for refined-bleached-deodorized low erucic acid rapeseed oil derived from canola-quality *Brassica juncea* (line PC97-03).

Property	Canola	PC97-03
Fatty Acids (relative weight %)		
<14	< 0.1	ND[1]
14 00	< 0 2	0 1
16.0	< 6.0	3 9
16.1	< 1 0	0 3
18.0	< 2.5	2.6
18 1	> 50.0	62.7
18 2	< 40.0	17 2
18 3	< 14 0	9.5
20:0	< 1.0	0.7
20:1	< 2.0	1.6
22 0	< 0 5	0 3
22·1	< 2 0	0 2
24.0	< 0 2	0 4
24:1	≤ 0 2	0.3
Acid Value	≤ 6	0 04
Cold Test (5.5 hour)	Passes test	Passes test
Color (AOCS-Wesson)	≤1 5R / 15Y	0 2R / 2 6Y
Erucic Acid	≤ 2 0%	0 2%
Free Fatty Acids (as oleic acid)	≤ 0 05%	0 02%
Heavy Metals as Pb[2]	≤ 5 mg/kg	< 1.53 mg/kg
Iodine Value	110 –126	110.5
Lead	< 0 1 mg/kg	< 0 1 mg/kg
Linolenic Acid	≤ 14%	9.5%
Peroxide Value	≤ 10 meq/kg	0 0 meq/kg
Refractive Index	1 465-1 467 at 40°C	1.465 at 40°C
Saponifiable Value	178-193	186 4
Stability (AOM)	≥ 7 h	22 0 h
Sulfur	≤ 10 mg/kg	0 62 mg/kg
Unsaponifiable Matter	≤ 1.5%	0 99%
Water	≤ 0 1%	0.01%

[1] None detected
[2] For PC97-03, total of Ag, As, Bi, Cd, Cu, Hg, Pb, Sn and Sb.

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SUBMISSION END

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