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CFSAN Note to the File

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To: Administrative Record, BNF No. 000162

Subject: Canola with transformation event NS-B5ØØ27-4 (NS-B5ØØ27-4 canola)

Keywords: Canola; *Brassica napus*; low erucic acid rapeseed; omega-3 long-chain polyunsaturated fatty acids (LCPUFAs); docosahexaenoic acid (DHA); eicosapentaenoic acid (EPA); fatty acid desaturase; fatty acid elongase; glufosinate ammonium herbicide tolerance; phosphinothricin N-acetyltransferase (PAT); OECD Unique identifier NS-B5ØØ27-4; Nuseed Americas, Inc.

Summary

Nuseed Americas, Inc. (Nuseed) has completed a consultation with the Food and Drug Administration (FDA) on food from NS-B5ØØ27-4 canola, with an altered oil composition and glufosinate ammonium herbicide tolerance. The altered oil composition is conferred through seed-specific expression of genes encoding fatty acid desaturases and elongases from yeast and marine microorganisms. Together, the introduced enzymes enable biosynthesis of long-chain polyunsaturated fatty acids (LCPUFAs), including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Glufosinate ammonium herbicide tolerance is conferred through constitutive expression of a gene encoding phosphinothricin N-acetyltransferase (PAT) from bacteria. This document summarizes Nuseed's conclusions and supporting data and information that FDA's Center for Food Safety and Applied Nutrition (CFSAN, we) evaluated pertaining to human food uses. FDA's Center for Veterinary Medicine summarizes its evaluation pertaining to animal food in a separate document.

Nuseed concludes:

- it has not introduced into human food a new protein or other substance that would require premarket approval as a food additive.
- oil from NS-B5ØØ27-4 canola is as safe for human food use as other oils that contain LCPUFAs and that are currently on the market, including menhaden oil; and, subject to the limitations in 21 CFR 184.1472, is generally recognized as safe (GRAS) for use in human food.[1]

[1] The Generally Recognized as Safe (GRAS) affirmation regulation for menhaden oil (21 CFR 184.1472) establishes limits on the use of menhaden oil to avoid total dietary exposures above 3.0 grams per person per day (g/p/d) of EPA and DHA combined. Paragraph (a)(3) limits maximum use levels of menhaden oil in specific food categories; paragraph (a)(4) restricts use of menhaden oil in combination with any other added oil that is a significant source of EPA or DHA.

- solvent-extracted meal from NS-B5ØØ27-4 canola is comparable to and as safe for human food use as other solvent-extracted canola meals that are currently on the market.

CFSAN evaluated data and information supporting these conclusions and considered whether NS-B5ØØ27-4 canola raises other regulatory issues involving use in human food under the Federal Food, Drug, and Cosmetic Act (FD&C Act). We have no further safety, nutritional, or regulatory compliance questions at this time about Nuseed's current intended uses of NS-B5ØØ27-4 canola in human food.

Subject of the Consultation

Crop:	Canola (*Brassica napus*)
Designations:	NS-B5ØØ27-4
Intended trait:	Altered oil composition; biosynthesis of omega-3 LCPUFAs
Intended trait:	Tolerance to glufosinate ammonium herbicides
Developer:	Nuseed Americas, Inc.
Submission received:	April 25, 2017
Amendments received:	June 14, 2018 (amended submission); March 15, 2019; June 20 and 24, 2019; December 14, 2019; March 4, 2020; May 6, 2020; May 14, 2020; August 4, 2020; and October 28, 2020
Intended use:	Use of the oil subject to the limitations in 21 CFR 184.1472; general uses customary for solvent-extracted canola meal.
Transformation vector:	pJP3416_GA7-ModB[2]
Expression cassette 1:	The Lackl-Δ12D cassette encodes a delta-12 desaturase from *Lachancea kluyveri*. The Lackl-Δ12D enzyme catalyzes the conversion of 18:1 n-9 (oleic acid, OA) to 18:2 n-6 (linoleic acid, LA).

[2] The genes in the expression cassettes were chemically synthesized based on sequences originally identified in yeast, marine microorganisms, or bacteria. Although the DNA sequences were modified for expression in canola, the predicted amino acid sequence of their encoded proteins is unchanged.

Expression cassette 2:	The Picpa-ω3D cassette encodes an omega-3/delta-15 desaturase from *Pichia pastoris*. The Picpa-ω3/Δ15D enzyme catalyzes the conversion of 18:2 n-6 (LA) to 18:3 n-3 (α-linolenic acid, ALA).
Expression cassette 3:	The Micpu-Δ6D cassette encodes a delta-6 desaturase from *Micromonas pusilla*. The Micpu-Δ6D enzyme catalyzes the conversion of 18:3 n-3 (ALA) acid to 18:4 n-3 (stearidonic acid, SDA).
Expression cassette 4:	The Pyrco-Δ6E cassette encodes a delta-6 elongase from *Pyramimonas cordata*. The Pyrco-Δ6E enzyme catalyzes the conversion of 18:4 n-3 (SDA) to 20:4 n-3 (eicosatetraenoic acid, ETA).
Expression cassettes 5:	The Pavsa-Δ5D cassette encode a delta-5 desaturase from *Pavlova salina*. The Pavsa-Δ5D enzyme catalyzes the conversion of 20:4 n-3 (ETA) to 20:5 n-3 (eicosapentaenoic acid, EPA).
Expression cassettes 6:	The Pyrco-Δ5E cassette encode a delta-5 elongase from *P. cordata*. The Pyrco-Δ5E enzyme catalyzes the conversion of 20:5 n-3 (EPA) to 22:5 n-3 (docosapentaenoic acid, DPA).
Expression cassette 7:	The Pavsa-Δ4D cassette encodes a delta-4 desaturase from *P. salina*. The Pavsa-Δ4D enzyme catalyzes the conversion of 22:5 n-3 (DPA) to 22:6 n-3 (docosahexaenoic acid, DHA) and the conversion of 22:4 n-6 (docosatetraenoic acid; DTA) to 22:5 n-6.
Expression cassette 8:	The PAT cassette encodes the phosphinothricin N-acetyltransferase (PAT) from *Streptomyces viridochromogenes*. The PAT enzyme confers glufosinate ammonium herbicide tolerance.
Transformation method:	Agrobacterium-mediated transformation

Molecular Characterization

Confirmation of intended genetic change

Nuseed used a combination of three strategies to characterize the DNA insertions in NS-B5ØØ27-4 canola. First, Nuseed used vector-targeted polymerase chain reaction (PCR) and high-throughput sequencing to determine the DNA insert copy number, location, and integrity of plasmid DNA present in NS-B5ØØ27-4 canola genome. Next, Nuseed used whole genome sequencing to further characterize the DNA insertions. Genomic DNA was sequenced using high-throughput sequencing and reads were analyzed by alignment to the transformation

plasmid.[3] Based on the results of the first two strategies, Nuseed designed PCR primers to amplify the junctions between the DNA insertions and canola genome. The resulting amplicons were sequenced by Sanger sequencing.

Nuseed concludes from its analyses that two DNA inserts were integrated into the NS-B5ØØ27-4 canola genome on chromosomes A02 and A05 (hereafter referred to as Insert A02 and Insert A05). Insert A02 contained a single copy of the truncated T-DNA comprised of four complete gene expression cassettes (Picpa-ω3D, Pavsa-Δ5D, Pyrco-Δ5E, Micpu-Δ6D). Insert A05 contained two intact copies of the T-DNA, with each copy comprised of eight complete gene expression cassettes (PAT, Pyrco-Δ6E, Lackl-Δ12D, Pavsa-Δ4D, Picpa-ω3D, Pavsa-Δ5D, Pyrco-Δ5E, Micpu-Δ6D), linked by a short sequence from the T-DNA Left Border. According to Nuseed, the coding sequences of the DNA inserts matched the transformation vector reference sequences.

Nuseed identified two small deletions (≤ 20 base pairs) in the NS-B5ØØ27-4 canola genome: one at each of the two DNA insertion sites. Nuseed considered the potential impact of the deleted genome sequences and concludes these do not affect food safety based on the genomic location and on results from open reading frame and compositional analyses.

Absence of vector backbone DNA

Nuseed reports that sequencing results confirmed the NS-B5ØØ27-4 canola genome does not contain vector backbone DNA.

Open reading frame analysis

To identify potential open reading frames (ORFs) that may have resulted from insertion of the T-DNA into the canola genome, Nuseed analyzed the four junction sites between the DNA inserts and the canola genome as well as the junction site between the tandem copies of the T-DNA present in Insert A05. Nuseed identified 25 hypothetical "stop-to-stop" ORFs with deduced amino acid sequences ranging in length from 7 to 126 amino acids. Nuseed compared the deduced amino acid sequences to toxins and allergens in the National Center for Biotechnology Information (NCBI) Protein database and in version 16 of the Food Allergy Research and Resource Program (FARRP) Allergen Online database.[4] Nuseed reported that it did not identify any significant matches and therefore concludes that the development of NS-B5ØØ27-4 canola did not produce potential polypeptides that raise food safety concerns.

Stability over multiple generations

To determine the inheritance and stability of the genotype and phenotype, plant tissues from multiple segregating generations were screened. Copy number of the loci were estimated based on genetic markers specific to each insert. The phenotype was determined from measurement of LCPUFA levels. Chi-square analysis ($p = 0.05$) demonstrated that the inheritance pattern of the genotype and phenotype in the segregating populations was consistent with the inheritance of two independent loci according to Mendelian laws. Nuseed noted that both DNA sequencing

[3] According to Nuseed, high-throughput sequencing achieved at least 30x estimated depth of genome-wide coverage.

[4] Nuseed defined significant matches as sharing >35% identity over a sliding window of 80 amino acids or identity across 8 contiguous amino acids.

and junction-specific molecular analyses confirmed the stability of the two DNA inserts across multiple generations.

Introduced Proteins: fatty acid desaturases and elongases

Intended trait:	Altered oil composition; biosynthesis of omega-3 LCPUFAs
Source organisms:	• Yeast *(Lachancea kluyveri, Pichia pastoris*) • Marine microorganisms (*Micromonas pusilla*, *Pyramimonas cordata*, and *Pavlova salina*)
Protein descriptions:	Fatty acid desaturases catalyze the formation a double bond between two carbon atoms at specific positions on fatty acid carbon chains. The introduced desaturases are delta-12 desaturase, omega-3/delta-15 desaturase, delta-6 desaturase, delta-5 desaturase, and delta-4 desaturase. Fatty acid elongases catalyze the addition of an ethyl group at a fixed position on the fatty acid carbon chain, thereby extending its length by two carbons. The introduced elongases are delta-6 elongase and delta-5 elongase.
Intended function:	In combination, the introduced desaturases and elongases progressively desaturate and elongate fatty acids to produce the desired omega-3 LCPUFAs

Levels of introduced desaturases and elongases in NS-B5ØØ27-4 canola seed

Nuseed measured the levels of the introduced fatty acid desaturases and elongases in NS-B5ØØ27-4 canola seed using a liquid chromatography-multiple reaction monitoring-mass spectrometry (LC-MRM-MS) assay.[5] Nuseed reported that all seven introduced fatty acid desaturases and elongases were detected in both developing and mature seeds from NS-B5ØØ27-4 canola. In mature seed, the protein levels of the introduced fatty acid desaturases and elongases ranged from below the limit of quantitation (for Pyrco-Δ6E) up to a mean level of 1.55 µg/mg of total protein (for Pavsa-Δ4D). Based on these levels, Nuseed concludes that the introduced fatty acid desaturases and elongases represent a negligible portion of the total protein present in NS-B5ØØ27-4 canola seed.

Safety assessment of fatty acid desaturases and elongases in food

Nuseed relied on a weight of evidence approach to assess the safety of the introduced fatty acid desaturases and elongases in food derived from NS-B5ØØ27-4 canola, including information published on the sources and functional activities of the introduced fatty acid desaturases and elongases as well as on similar enzymes present in yeast, fungi, plants, and animals with a history of safe use in human or animal food, or in their production. Based on comparison of DNA insert sequencing results to transformation vector sequences, Nuseed concludes that the

[5] Study published in Colgrave et al., (2019). Quantitation of seven transmembrane proteins from the DHA biosynthesis pathway in genetically engineered canola by targeted mass spectrometry. *Food and Chemical Toxicology* **126**: 313-321.

amino acid sequences of the introduced fatty acid desaturases and elongases are predicted to be identical to the sequences originally identified in the source organisms. Nuseed cited published studies characterizing the functionality of the fatty acid desaturases and elongases in different heterologous expression systems and reported the results of its enzyme activity studies. With the exception of Pavsa-4ΔD, Nuseed expressed the desaturases and elongases individually in the yeast *P. pastoris* and measured their ability to desaturate or elongate specific fatty acids; Nuseed summarized the results of a published study characterizing Pavsa-4ΔD functionality in the yeast *Saccharomyces cerevisiae*.[6]

Nuseed used bioinformatic analysis to compare the amino acid sequences of the introduced fatty acid desaturases and elongases to the sequences of known toxins and allergens in the NCBI Protein and FARRP databases.[7] Nuseed reported that it did not identify significant sequence matches between the proteins in these databases and the introduced fatty acid desaturases and elongases.

Nuseed measured the sensitivity of the introduced fatty acid desaturases and elongases to enzymatic digestion and to heat. Nuseed used recombinant fatty acid desaturases and elongases purified from either insect cell or bacterial expression systems as proxies for the proteins expressed in NS-B5ØØ27-4 canola. The sensitivity of the purified fatty acid desaturases and elongases to enzymatic digestion was measured by monitoring the appearance of peptide fragments using liquid chromatography tandem mass spectrometry (LC-MS/MS) analyses following incubation with pepsin or with pepsin plus trypsin. Nuseed determined that the fatty acid desaturases and elongases were readily digestible in pepsin (≥ 75% within 10 minutes and ≥90% within 60 minutes).[8] The sensitivity of the desaturases and elongases to heat was measured by estimating thermal denaturation (aggregation) following exposure to various temperatures for up to 30-minute periods.[9] Nuseed determined that the desaturases and elongases showed considerable aggregation when incubated for 30 minutes at 95°C: six of the proteins showed less than 15% remained unaggregated or soluble; the Lackl-Δ12D enzyme showed 36% remained unaggregated or soluble. Nuseed concludes that these values represent an upper estimate of protein levels remaining in soluble form. Nuseed anticipates little to no functional activity from these proteins after processing of the canola seed into meal and oil.

Based on the weight of this evidence, Nuseed concludes that the introduced fatty acid desaturases and elongases do not raise any safety concerns with regard to human health.

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[6] Zhou et al., (2007). Isolation and characterization of genes from the marine microalga *Pavlova salina* encoding three front-end desaturases involved in docosahexaenoic acid biosynthesis. *Phytochemistry* **68**: 785-796.

[7] Nuseed used the FARRP Allergen Online database to identify sequence similarities to allergens of > 35% identity over 80 amino acids on a sliding window or 100% identity over 8 contiguous amino acids. Nuseed used the NCBI Protein database to identify sequence similarities to toxins and allergens of ≥ 50%.

[8] Study published in Colgrave et al., (2019). Proteomics reveals the *in vitro* protein digestibility of seven transmembrane enzymes from the docosahexaenoic acid biosynthesis pathway. *Food and Chemical Toxicology* **130**: 89-98.

[9] Nuseed explained that it used an insect cell-based protein expression system to produce the fatty acid desaturases and elongases for the *in vitro* thermal stability assays.

Introduced Protein: PAT

Intended trait:	Tolerance to glufosinate ammonium herbicide
Source organism:	Bacteria (*Streptomyces viridochromogenes*)
Intended function:	PAT catalyzes the acetylation of glufosinate ammonium herbicide

Levels of PAT in NS-B5ØØ27-4 canola seed

Nuseed measured the levels of PAT in developing and mature seed from NS-B5ØØ27-4 canola using an LC-MRM-MS assay. Nuseed reported that PAT was detected in both developing and mature seeds. The mean level in the mature seed ranged from 23 to 33 ng PAT/mg total protein.[5] Nuseed concludes that PAT represents a negligible portion of the total protein present in NS-B5ØØ27-4 canola seed.

Safety assessment of PAT in human food

Based on comparison of DNA insert sequencing results to transformation vector sequences, Nuseed stated that the amino acid sequences of the PAT protein expressed in NS-B5ØØ27-4 canola is predicted to be identical to the PAT proteins expressed in other genetically engineered crops that have been evaluated for their safe use in human food, citing several completed FDA consultations.[10] Nuseed also summarized a published study by Hérouet et al., (2005), in which the authors provided evidence that PAT is substrate specific, does not share sequence or functional similarities with known toxins or allergens, is digested in simulated gastric and intestinal fluids, and loses enzymatic activity within 10 minutes at temperatures of ≥ 60˚C. Based on the weight of this evidence and on the low levels of PAT in mature NS-B5ØØ27-4 canola seed, Nuseed concludes that there are no food safety concerns associated with the expression of PAT in NS-B5ØØ27-4 canola seed.

Intended Human Food Uses

Canola refers to varieties of rapeseed (of species *B. napus*, *B. rapa*, and *B. juncea*) that are low in erucic acid and glucosinolates.[11] Canola is used primarily as a source of edible seed oil. Canola seed also has minor uses as a source of protein isolates and lecithin.[12] NS-B5ØØ27-4 canola (*B. napus*) has been genetically engineered to enable biosynthesis of LCPUFAs, including EPA and DHA, that would not otherwise be present in canola oil.

The omega-3 LCPUFAs EPA and DHA are commonly found in some fish oils, including menhaden. Menhaden oil is the subject of a GRAS Affirmation regulation (21 CFR 184.1472; menhaden regulation) that includes limitations on the use of menhaden oil in food to avoid dietary exposures above 3.0 grams per person per day (g/p/d) of EPA and DHA. The regulation limits the use of menhaden oil to food categories listed in 21 CFR 184.1472(a)(3). These categories include but are not limited to baked goods; cereals and pastas; confections and candy;

[10] Nuseed cited Biotechnology Notification Files (BNFs) 46, 55, and 136.
[11] See definitions in 7 CFR 810.301 for canola seed and 21 CFR 184.1555(c) for canola oil.
[12] See GRAS Notice Nos. (GRNs) 327, 386, 533, 682, and 683.

egg, fish, meat, and milk products; desserts and snack foods; dressings and spreads; gravies and sauces; and (non-alcoholic) beverages. The regulation also limits the use level of menhaden oil in each food category. The maximum levels of use in 21 CFR 184.1472(a)(3) are based on the composition of menhaden oil (13% EPA and 7% DHA) and the targeted dietary exposure limit of 3.0 g/p/d EPA and DHA. To further ensure the safe use of menhaden oil, 21 CFR 184.1472(a)(4) states that it shall not be used in combination with any other added oil that is a significant source of EPA or DHA.

Likewise, other fish[13] and DHA-containing algal[14] oils have been determined to be GRAS for uses in human food where the uses are substitutional for those listed in the menhaden regulation such that (1) the maximum levels of use are based on the EPA and DHA content of the oil and the targeted dietary exposure limit of 3.0 g/p/d of EPA and DHA and (2) the oil will not be used in combination with any other added oil that is a significant source of EPA or DHA. Nuseed explains that NS-B5ØØ27-4 canola oil (containing an approximate range of 7-11% EPA plus DHA) is intended to be used in human food as a substitute for EPA- and DHA-containing oils, including menhaden oil and other fish and algal oils. Nuseed concludes that oil from NS-B5ØØ27-4 canola is GRAS under its intended conditions of use in human food within the limitations of 21 CFR 184.1472.

Human Food Nutritional Assessment

With the exception of altering the seed oil composition, the DNA insertions in NS-B5ØØ27-4 canola are not expected to change levels of key nutrients, anti-nutrients, or toxicants. To assess the intended changes in seed oil composition as well as potential unintended changes in other key components relevant to safety or nutrition, Nuseed conducted eight field trials at multiple sites in major canola growing regions of Australia during the 2015 season. Nuseed collected and analyzed mature seed from NS-B5ØØ27-4 canola, the parental variety AV Jade (control), and seven commercial varieties (reference). Nuseed measured levels of proximates and fiber, fatty acids, amino acids, vitamins, minerals, phytosterols and anti-nutrients in whole seed.[15] Nuseed also measured levels of key components in oil, crude meal, and solvent-extracted meal. The analytical results from the NS-B5ØØ27-4, control, and reference varieties were compared to each other and to published data and information.

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[13] The GRAS status of certain fish oils when used in food in accordance with the menhaden oil regulation has been determined in GRAS Notices receiving "no questions" letters from FDA. These include salmon oil (GRN 146), fish oil-predominantly anchovy (GRNs 138, 193), and tuna oil (GRN 109).

[14] The GRAS status of oils from the algae *Schizochytrium* sp. when used in food in accordance with the menhaden oil regulation has been determine in GRAS notices receiving "no questions" letters from FDA. These include GRNs 137 and 732.

[15] The components selected for analysis in NS-B5ØØ27-4 canola are consistent with those identified in the Organisation for Economic Co-operation and Development (OECD) 2011 Revised Consensus Document on Compositional Considerations for New Varieties of Low Erucic Acid Rapeseed (Canola): Key Food and Feed Nutrients, Anti-Nutrients and Toxicants. OECD Environment, Health and Safety Publications Series on the Safety of Novel Foods and Feeds, No. 24. ENV/JM/MONO(2011)55.

Analysis of seed oil

Characterization of NS-B5ØØ27-4 canola oil composition

Nuseed reported the levels of individual fatty acids in seed from NS-B5ØØ27-4, control, and reference canola varieties on a percentage of total fatty acids (% TFA) basis. Nuseed compared NS-B5ØØ27-4 canola oil composition to the composition of edible oils ordinarily consumed in the human diet using data and information available in the ILSI Crop Composition Database,[16] Codex Standards for Named Vegetable Oils[17] and for Fish Oils,[18] FDA's GRAS Notice Inventory,[19] and published literature. The composition of NS-B5ØØ27-4 canola oil was initially compared to the composition of oils from commercial canola varieties. Based on this comparison, the fatty acids in NS-B5ØØ27-4 canola seed oil fell into three categories: (1) endogenous fatty acids *unaffected* by the altered metabolic pathway, (2) endogenous fatty acids *affected* by the altered metabolic pathway, and (3) polyunsaturated fatty acids *introduced* as a result of the altered metabolic pathway. Nuseed subsequently compared the composition of NS-B5ØØ27-4 canola oil to the composition of other oils with histories of safe use in human food (hereafter "LCPUFA-containing oils" including fish oils and *Schizochytrium sp.* algal oils).

[16] International Life Science Institute (2016) Crop Composition Database Version 6.0. The ILSI database is now known as the Agriculture and Food Systems Institute (AFSI) Crop Composition Database; accessible at https://www.cropcomposition.org.
[17] Values for low erucic acid rapeseed (LEAR) oil in the Codex Standard for Named Vegetable Oils CODEX STAN 210-1999. Codex standard values are for sum of isomers.
[18] Values for menhaden, salmon (farmed), and anchovy oils in Codex Standard for Fish Oils CODEX CXS 329-2017. Codex standard values are for individual isomers, where detectable.
[19] Accessible at https://www.fda.gov/grasnoticeinventory.

The fatty acids in NS-B5ØØ27-4 canola unaffected by the altered metabolic pathway include fatty acids with values that were reliably within the ranges observed for the control and/or reference canola varieties;[20] fatty acids that had one or more values outside control and reference ranges but were consistent with literature ranges;[21] fatty acids present at levels greater than the Limit of Quantitation (LOQ) but at low concentrations (≤0.2%) in NS-B5ØØ27-4, control, and reference canola varieties, consistent with literature ranges;[22] and fatty acids that were below the LOQ in NS-B5ØØ27-4 canola, consistent with literature ranges.[23] Nuseed concludes that fatty acids unaffected by the altered metabolic pathway do not raise nutritional or safety concerns because their levels are comparable to levels in canola varieties with a history of safe use.

Table 1 - Endogenous canola fatty acids unaffected by the altered metabolic pathway (% TFA)

Fatty acid	NS-B5ØØ27-4 canola oil mean (range)	Reference canola varieties range	Codex canola oil range[17]	Codex fish oils combined range[18] [18]
16:0 (palmitic)	4.5 (4.4-4.7)	3.6-4.8	2.5-7.0	6.5-22.0
16:1 n-7	0.19 (0.18-0.21)	0.15-0.25	--	2.0-13.0
16:1 sum	0.29 (0.27-0.33)	0.21-0.34	ND-0.6	--
18:0 (stearic)	2.2 (2.0-2.5)	1.4-2.3	0.8-3.0	1.0-7.0
18:1 n-7 (vaccenic)	2.9 (2.7-3.1)	2.4-3.1	--	1.7-3.7
20:0 (arachidic)	0.59 (0.57-0.62)	0.42-0.73	0.2-1.2	ND-1.8
20:1 n-9 (gondoic)	1.2 (1.1-1.3)	0.88-1.6	--	ND-7.0
20:1 sum	--	--	0.1-4.3	--
22:0 (behenic)	0.25 (0.24-0.27)	0.18-0.39	ND-0.6	--

Fatty acids present at ≤0.2% TFA (14:0, 16:1 n-9, 17:0, 17:1, 20:2 n-6, 24:0, 24:1 n-9) are not shown. -- value not available; Codex non-detectable (ND) values defined as ≤0.05%.

The fatty acids in NS-B5ØØ27-4 canola affected by the altered metabolic pathway were those that have mean values consistently outside the range of the reference varieties and literature values for conventional canola. The introduced fatty acid desaturases and elongases enable production of LCPUFAs through the desaturation and elongation of their endogenous precursors, including oleic acid (OA), linoleic acid (LA), and α-linolenic acid (ALA); accordingly, changes in the levels of these fatty acids were not unexpected. The level of OA in oil from NS-B5ØØ27-4 canola (mean 42%) was lower compared to the range of OA observed in the reference

[20] including 14:0 (myristic), 16:0 (palmitic), 16:1 n-7 (palmitoleic), 18:1 n-7 (vaccenic), 20:0 (arachidic), 20:1 n-9 (gondoic), 22:0 (behenic), and 20:2 n-6
[21] including 18:0 (stearic)
[22] including 16:1 n-9, C17:0, 17:1, 24:0 (lignoceric) and 24:1 n-9 (nervonic)
[23] including 20:2 n-9, 22:1 n-9 (erucic), and 22:2 n-6

canola varieties and reported for canola oil in the Codex Standard for Named Vegetable Oils; but it was still the predominant fatty acid in NS-B5ØØ27-4 canola oil, and within the range of Codex standard reference values for fish oils, for which NS-B5ØØ27-4 canola oil is intended to be a substitute. For the essential fatty acids, the level of LA in NS-B5ØØ27-4 canola oil (8.5%) was lower while the level of ALA in NS-B5ØØ27-4 canola oil (21.0%) was higher compared to reference canola varieties and Codex standard reference values for canola. Both LA and ALA were present in NS-B5ØØ27-4 canola oil at levels comparable to or higher than the range of Codex standard reference values for several fish oils, for which NS-B5ØØ27-4 canola oil is intended to substitute.

Table 2 - Endogenous canola fatty acids affected by the altered metabolic pathway (% TFA)

Fatty acid	NS-B5ØØ27-4 canola oil Mean (range)	Reference canola varieties	Codex canola oil[17]	Codex fish oil combined range[18]
18:1 n-9 (oleic)	42.0 (37.2-47.4)	49.2-72.7	--	3.6-47
18:1 sum	45.0 (40.4-50.3)	51.9-74.4	51-70	--
18:2 n-6 (linoleic)	8.5 (8.0-9.1)	11.6-23.3	--	ND-15.0
18:2 sum	8.8 (8.4-9.3)	11.7-23.4	15-30	--
18:3 n-3 (α-linolenic)	21.0 (18.8-22.9)	3.9-12.1	--	ND-7.0
18:3 sum	22.2 (19.8-24.2)	3.9-12.2	5.0-14	--

Fatty acids present at ≤0.2% of fatty acids are not shown. -- value not available; Codex non-detectable (ND) values defined as ≤0.05%.

The fatty acids introduced in NS-B5ØØ27-4 canola as a result of the altered metabolic pathway include those fatty acids that were not present in commercial canola varieties described in the literature and that were not listed for canola oil in the ILSI Crop Composition Database[16] or the Codex Standard for Named Vegetable Oils.[17] The introduced fatty acids include the PUFAs γ-linolenic (GLA), and stearidonic (SDA), and the LCPUFAs eicosatrienoic (ETrA), eicosatetraenoic (ETA), eicosapentaenoic (EPA), 22:4 n-3, docosapentaenoic (DPA), osbond, and docosahexaenoic (DHA). Nuseed confirmed that omega-6 LCPUFAs that were not expected to be introduced by the altered metabolic pathway, such as 20:3 n-6 (dihomo-γ-linolenic), 20:4 n-6 (arachidonic), 22:2 n-6, and 22:4 n-6, were below the limit of quantitation[24] in the NS-B5ØØ27-4 canola oil. Nuseed noted that the fatty acids introduced in NS-B5ØØ27-4 canola oil are not new to the human diet but are ordinarily present in edible fish[13] and algal[14] oils for which NS-B5ØØ27-4 canola oil is intended to substitute. For comparison, values for farmed salmon and menhaden oils from the Codex Standard for Fish Oils and for *Schizochytrium* sp. algal oils are shown in Table 3.

Of the omega-3 LCPUFAs introduced in NS-B5ØØ27-4 canola, Nuseed reported that DHA (8.4%) was present at the highest level while EPA (0.4%) was present at a lower level. This is

[24] LOQ=0.01% for fatty acids in NS-B5ØØ27-4 canola oil.

consistent with the ratio of DHA to EPA in S*chizochytrium* sp. algal oils containing DHA. The combined level of DHA and EPA in NS-B5ØØ27-4 canola oil (ranging from 7-11%) was similar to the level in farmed salmon oil but lower than in both menhaden oil and *Schizochytrium* sp. algal oils. Nuseed noted that the fatty acid intermediates, SDA, ETA, and DPA, were also present in NS-B5ØØ27-4 canola oil at levels that were generally 1-3%, consistent with levels reported in fish oils. Low levels of 20:3 n-3 and 22:4 n-3 in NS-B5ØØ27-4 canola oil are consistent with published values for fish oils (not shown in Table 3).

Table 3 - Fatty acids introduced as a result of the altered metabolic pathway (% TFA)

Fatty acid	**NS-B5ØØ27-4 canola oil Mean (range)**	**Codex farmed salmon oil[18]**	**Codex menhaden oil[18]**	***Schizochytrium* sp. algal oil combined range[14]**
18:3 n-6 (GLA)	0.45 (0.38-0.55)	ND-0.5	ND-2.5	0.1
18:4 n-3 (SDA)	2.5 (2.0-3.2)	0.5-1.5	1.5-3.0	Trace-0.9
20:3 n-3 (ETrA)	0.58 (0.46-0.67)	--	--	1.3-2.4
20:4 n-3 (ETA)	1.1 (1.0-1.3)	0.5-1.0	--	0.8-0.9
20:5 n-3 (EPA)	0.44 (0.32-0.52)	2.0-6.0	12.5-19.0	0.7-3.2
22:4 n-3	0.23 (0.20-0.24)	--	--	--
22:5 n-3 (DPA)	1.1 (0.80-1.2)	1.0-2.5	2.0-3.0	0.1
22:5 n-6 (osbond)	0.09 (0.07-0.11)	--	--	10.3-15.0
22:6 n-3 (DHA)	8.4 (6.5-10.3)	3.0-10.0	5.0-11.5	32.5-50.7
EPA+DHA	8.8 (6.8-10.8)	5.0-16.0	17.5-30.5	33.2-53.9

Upper levels of 20:3 n-3 in *Schizochytrium sp.* oil may reflect co-elution with 20:4 n-7. -- value not available; Codex non-detectable (ND) values defined as ≤0.05%.

In summary, consistent with the altered fatty acid pathway, NS-B5ØØ27-4 canola oil contains lower levels of total monounsaturated fatty acids (MUFAs) and higher levels of total polyunsaturated fatty acids (PUFAs) than conventional canola oil. The MUFA and PUFA levels in NS-B5ØØ27-4 canola oil are within the ranges observed in other common vegetable oils. The total omega-6 fatty acids in NS-B5ØØ27-4 canola are lower than that present in conventional canola; however, these levels are at or above that present in fish oils. The introduced LCPUFAs – which include ETrA, ETA, EPA, 22:4 n-3, DPA, 22:5 n-6, and DHA – make NS-B5ØØ27-4 canola oil qualitatively similar to LCPUFA-containing oils currently in the human diet. The total level of EPA+DHA (omega-3 LCPUFAs) in NS-B5ØØ27-4 canola oil is approximately one-half that of menhaden oil, although it is comparable to the total level of EPA+DHA in farmed salmon oil. In summary, the total level of omega-3 LCPUFAs in NS-B5ØØ27-4 canola oil, is within the range of omega-3 LCPUFAs in fish oils but not as high as in some oils currently in the human diet.

Safety assessment of NS-B5ØØ27-4 canola oil

Nuseed used a weight of evidence approach to assess the safety of the altered oil composition. Nuseed considered data and information about the composition and use of LCPUFA-containing oils available in published literature, the 1997 menhaden oil final rule[25] and regulation,[1] and FDA's GRAS Notice Inventory.[19] Nuseed concludes that the fatty acids in NS-B5ØØ27-4 canola oil – including the introduced fatty acids – are currently consumed in human diets and are present at levels that are comparable to those found in other consumed oils.

NS-B5ØØ27-4 canola oil contains the omega-3 LCPUFAs EPA and DHA, which are present in menhaden oil and known to have physiologic effects on bleeding time, glycemic control, and LDL cholesterol levels. FDA affirmed the GRAS status of menhaden oil with limitations on its use to avoid dietary intakes above 3.0 g per person per day (g/p/d) of EPA and DHA.[25] In determining the limitations on use of menhaden oil and the resulting estimated dietary exposure to EPA and DHA from use of menhaden oil, FDA cited a mean combined level of approximately 20% EPA and DHA in menhaden oil. Nuseed compared the combined level of EPA and DHA in NS-B5ØØ27-4 canola oil to the levels in menhaden oil and other EPA- and DHA-containing oils for which use in food has been determined as GRAS. Nuseed reported that the combined level of EPA and DHA in NS-B5ØØ27-4 canola oil (approximately 10%) is lower compared to menhaden oil as cited in the 1997 final rule, although DHA levels are within the ranges of DHA found in various fish oils as reported in the Codex Standard for Fish Oils[18] and in several GRAS Notices.[13, 14] Based on the compositional comparison with menhaden oil, Nuseed determined that NS-B5ØØ27-4 canola oil would provide approximately half of the total amount of EPA plus DHA if used as a 1:1 (by volume) replacement for menhaden oil in human food.

Nuseed estimated dietary exposure of EPA and DHA from the intended use of NS-B5ØØ27-4 canola oil as a 1:1 replacement for menhaden oil in human food. Nuseed used an estimated dietary exposure of 2.8 g/p/d of DHA and EPA from menhaden oil (See 62 FR 30751 at 30754), which corresponds to a dietary exposure of approximately 14 g/p/d of menhaden oil based on its use at the maximum use levels in the food categories specified in the menhaden oil regulation.[26] Use of NS-B5ØØ27-4 canola at 14 g/p/d in the total diet provides approximately 1.5 g/p/d EPA and DHA combined, for oil containing up to 0.5% EPA and 10.3% DHA. Thus, the estimate of dietary exposure to EPA and DHA is below the limit of 3.0 g/p/d targeted in the menhaden oil regulation when NS-B5ØØ27-4 canola oil is used as a 1:1 replacement for menhaden oil, the use of which is limited by 21 CFR 184.1472 (3) and (4).

Nuseed concludes, based on scientific procedures and considering publicly available information referenced in its safety and nutritional assessment, that oil from NS-B5ØØ27-4 canola is GRAS under its intended conditions of use in human food within the limitations of 21 CFR 184.1472.

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[25] Final Rule Substances Affirmed as Generally Recognized as Safe: Menhaden Oil (62 FR 30751; June 5, 1997).

[26] An updated estimate of mean dietary exposure of 2.7 g/p/d of EPA and DHA for consumers ages 2 years and older was provided in the 2002 proposed rule amending 21 CFR 184.1472 (67 FR 8744; February 26, 2002); this estimate corresponds to approximately 13 g/p/d of menhaden oil reflecting a minor difference in the exposure estimates between the original and revised uses.

Analysis of other key components

Aside from expected changes in fatty acid composition, Nuseed observed differences between NS-B5ØØ27-4 canola and the control in the levels of key components in seed, crude meal, and solvent-extracted meal. However, Nuseed explains that the differences were small and the mean levels of all components in NS-B5ØØ27-4 were within the range of values obtained for the reference varieties and/or the range of values reported in the ILSI Crop Composition Database.[16] NS-B5ØØ27-4 canola seed contains low levels of erucic acid and glucosinolates, consistent with quality standards for food-grade canola.[11] Nuseed states that no consistent pattern emerged to suggest that biologically significant changes in composition or nutritive value of the seed had occurred as an unexpected result of the transformation process. Nuseed concludes that NS-B5ØØ27-4 canola is compositionally comparable to conventional canola, except for its altered fatty acid composition.

Human Food Labeling Considerations

It is a producer's or distributor's responsibility to ensure that labeling of the foods it markets derived from NS-B5ØØ27-4 canola meets applicable legal requirements, including disclosure of any material differences (for example, differences in function, composition and nutritional or safety profiles) in the food as compared to its conventional counterpart. It is our understanding that NS-B5ØØ27-4 canola may be used in various food applications. Depending on the particular food application, the altered fatty acid composition of the oil may be considered a material fact requiring disclosure under Sections 201(n) and 403(a)(1) of the FD&C Act. Companies marketing oil from NS-B5ØØ27-4 canola or products containing oil from NS-B5ØØ27-4 canola are advised to consult with CFSAN's Office of Nutrition and Food Labeling, Division of Food Labeling and Standards, to discuss any required or voluntary labeling including statements relating to attributes of this canola variety and products produced from it. Failure to do so may result in the misbranding of products produced from NS-B5ØØ27-4 canola within the meaning of Sections 201(n) and 403(a)(1) of the FD&C Act.

Conclusion

Based on the information provided by Nuseed and other information available to CFSAN, we have no further safety, nutritional, or regulatory compliance questions at this time about Nuseed's current intended uses of NS-B5ØØ27-4 canola in human food. We consider the consultation with Nuseed on NS-B5ØØ27-4 canola to be complete.

Carrie H. Mcmahon -S

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Carrie McMahon, Ph.D.