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NUSEED NUTRITIONAL

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By llightle at 11:05 am, Dec 18, 2023

15 December 2023

Bernadette Juarez
U.S. Department of Agriculture
APHIS Deputy Administrator
Biotechnology Regulatory Services

Contains Confidential Business Information – 23-228-01

Dear Ms. Juarez,

Nuseed Nutritional US Inc, (herein referred to as "Nuseed"), a wholly owned subsidiary of Nufarm Limited, is submitting a revised request for Regulatory Status Review of canola genetically engineered to produce EPA and other long chain omega-3 fatty acids regulation under USDA APHIS 7 CFR part 340 as per the request from Mr. Tangredi on 7 Dec 2023.

The subject of this revision is to update the Confidential Business Information (CBI) captioning and justification pertaining to the review of the construct for production of EPA in canola. These updates are not expected to impact the ongoing scientific review of this request.

CBI Justification

Information marked by Nuseed as CBI is exempt from release and/or public disclosure under Freedom of Information Act (FOIA) Exemption 4, 5 U.S.C. Section 552(b)(4), which states that commercial and financial information that is both customarily and actually treated as private by its owner and is provided to the government under an assurance of privacy, thereby marked as "CBI". Release of information marked as CBI would provide competitive information about the nature of the research, development, and commercialization plans of Nuseed and could jeopardize protection of its intellectual property rights. Access to this information would cause competitive and/or financial harm to Nuseed.

If you have any questions pertaining to this request, feel free to contact me.

Best regards,

Mike Connelly

Regulatory Affairs
Nuseed Nutritional
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Information Supporting a Regulatory Status Review of Canola Genetically Engineered to Produce EPA and other Long Chain Omega-3 Fatty Acids

Submitted on behalf of:

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Originally submitted on

15 August 2023

Revisions
5 October 2023
15 December 2023

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Introduction

Nuseed Nutritional US Inc, (herein referred to as "Nuseed"), a wholly owned subsidiary of Nufarm Limited, is submitting information to USDA APHIS to support a Regulatory Status Review for canola (*Brassica napus*) that has been genetically engineered to produce oil containing omega-3 long-chain (≥C20) polyunsaturated fatty acids (ω3 LCPUFAs), with a significant amount of eicosapentaenoic acid (EPA, 20:5 ω3).

The ω3 LCPUFAs EPA and docosahexaenoic acid (DHA, 22:6ω3) are nutrients widely recognised for their roles as essential components of cell membranes and of particular importance for cardiovascular, cognitive and inflammatory health (Simpolous, 2006). EPA and DHA are primarily supplied from wild-caught fish and algal oils. However, marine sources are under pressure due to increasing demand for ω3 LC-PUFA by aquaculture, food, nutraceutical and pharmaceutical applications. Additional and sustainable sources of these fatty acids can be produced by engineering land-based oilseed crops to convert native fatty acids to marine-type ω3 LC-PUFA which are then accumulated in seed oil.

The subject of this request is for Regulatory Status Review, as per 7 CFR Section 340 of the genetically engineered EPA **[ ]**, containing five introduced fatty acid genes, for production of EPA in canola. These genes are identical to those present in the DHA canola event APHIS has already reviewed and concluded the canola and progeny derived from it are unlikely to pose a plant pest risk and therefore are no longer subject to the Regulations under 7 CFR part 340. Accordingly, APHIS concluded nonregulated status for DHA canola. Nuseed also submitted a food and feed safety and nutritional assessment of DHA Canola to US FDA as part of a Biotechnology Notification Consultation (BNF 162), FDA concluded the safety review with "no further questions". **CBI-del**

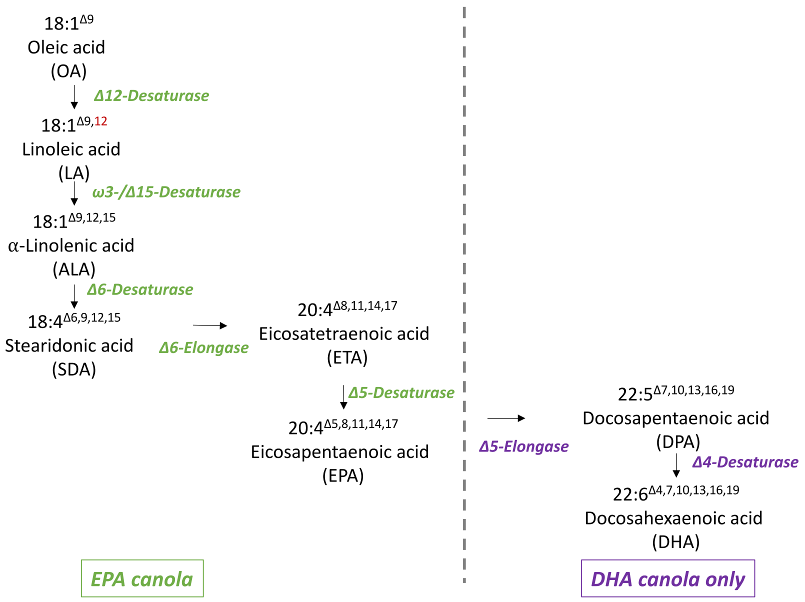

Figure 1. Biosynthesis pathway of **[ ]** (EPA canola) and NS-B5ØØ27-4 (DHA canola). **CBI-del**

EPA canola can be considered a subset of the deregulated DHA canola as it expresses the same pathway but with two fewer fatty acid enzymes, ending at the production of EPA (**Figure 1**). For your review, a summary of information on the comparator recipient plant, genotype of EPA canola, with emphasis on the long chain omega-3 fatty acid pathway genes, their mechanism of action (MOA) and functions, the sequence of **[ ]** T-DNA construct and phenotype of the trait are provided in this submission. **CBI-del**

Useful Definitions

NS-B50027-4 DHA canola event, produced via transformation of **pJP3416_GA7-ModB** construct, contains full set of 8 expression cassettes, produces 7 new omega-3 fatty acids in seed oil, previously granted nonregulated status by APHIS.
[ ], being used to transform canola for EPA production. **CBI-del**

1 Description of *Brassica napus* (Canola), the comparator plant

Scientific name: *Brassica napus* L.
Family: *Brassicacea*
Genus: *Brassica*
Species: *napus* (2N=38)
Common name: canola, oilseed rape, rapeseed

Traditional rapeseed has been cultivated in Asia, Europe and North-western Africa since ancient times as a source of oil for lamps, soap and later for industrial purposes, but was unsuitable as a food source for humans and animals due to its naturally occurring erucic acid and glucosinolate content (CFIA, 2017). An edible version, canola, was developed and has been grown and consumed since the 1980's.

The international regulatory standard for canola meets the following USDA and OECD standard definition:

> "Seeds of the genus Brassica (*Brassica napus*, *Brassica rapa* or *Brassica juncea*) from which the oil shall contain less than 2% erucic acid in its fatty acid profile and the solid component shall contain less than 30 micromoles of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3 butenyl glucosinolate, and 2-hydroxy- 4-pentenyl glucosinolate per gram of air-dry, oil-free solid." (USDA, 1992; OECD, 2011).

Reference documents for *B. napus* L. are available from national and international organizations (OGTR, 2017; OECD, 2012, CFIA), and provide additional background on its biology, including:

- information on use of canola as a crop plant
- taxonomic status of Brassica
- identification methods
- reproductive biology
- centers of origin and diversity
- crosses, including intra-and inter-specific/genus crosses and gene flow
- agro-ecology, including information about cultivation, volunteers and weediness, soil ecology, and canola-insect interactions.

Canola is among the top oilseed crops in the world. Canola seed is processed into two main fractions: oil and meal, both of which have a history of safe use in human food and animal feed (OECD, 2011) and are Generally Recognized As Safe (GRAS) as a food or feed component (https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm? fr=184.1555). Canola oil is commonly used for cooking/frying and in food products such as margarines and salad dressings, along with a vast array of ready-to-eat and processed foods. Canola oil is also used as industrial oil and lubricants as well as a source of biodiesel. Canola meal is widely used as an animal feed for cattle, swine and poultry.

EPA canola oil can be used for human food and dietary supplements and for aquaculture feed.

2 Genotype of EPA canola

Origin and functions of the inserted genes

EPA canola is developed using *Agrobacterium*-mediated transformation of a conventional canola variety with the **[ ]** vector to introduce the EPA biosynthetic pathway resulting in production of EPA and other long chain omega-3 fatty acids. The genes, their functions and sources are described in **Table 1**. **[** **CBI-del**

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Figure 2. Physical map of binary vector [ ] **CBI-del**

The gene cassettes were synthesized for *Brassica* species and intentionally matched to the amino acid sequence of the native genes from the source organisms, which were used to develop the EPA construct. The EPA oil in EPA canola will be identical to the EPA expressed in other deregulated events, such as Nuseed's DHA canola (17-236-01p) and BASF's EPA and DHA (LBFLFK) canola (17-321-01p) as well as EPA naturally occurring in marine sources (i.e., fish species).

The EPA synthetic pathway therefore consists of oleic acid (OA) being converted to linoleic acid (LA) by the desaturase Lackl-Δ12D, LA is desaturated to α-linolenic acid (ALA) by Picpa-ω3D, ALA to stearidonic acid (SDA) by Micpu-Δ6D, SDA is converted to eicosatetraenoic acid (ETA) by Pyrco-Δ6E, and finally ETA to EPA by Pavsa-Δ5D. All genes are regulated by seed-specific promoters. This pathway represents the same MOA as was used for DHA canola minus the last two steps of converting EPA to DPA then DPA to DHA. The identical seed-specific gene cassette (promotor, coding sequence and terminator) present in DHA canola is used for EPA canola.

As with DHA canola, EPA canola expresses the glufosinate herbicide-resistant trait resulting from the expression of the *pat* gene that encodes the enzyme phosphinothricin-N-acetyltransferase (PAT). The PAT enzyme works by acetylating L-phosphinothricin, which is the active isomer of glufosinate herbicides, rendering the crop resistant to application of this non-selective herbicide during the growing season.

A summary of pJP3416_GA7-ModB (DHA canola) and **[ ]** constructs, gene expression cassettes and their functions are provided in **Table 1**. Genetic elements in the T-DNA of the **[ ]** transformed into the B. napus genome are described in **Table 2.** **CBI-del** **CBI-del** **CBI-del**

Table 1. Comparison of pJP3416_GA7-ModB and **[ ]** constructs to develop EPA Canola **CBI-del**

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Table 2. Description of the genetic elements of **[ ]** for *B. napus* transformation **CBI-del**

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3 Sequence of the EPA Construct

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4 Intended Trait, Mechanism of Action and Phenotype of EPA Canola

EPA canola is being developed to produce the long chain omega-3 fatty acid (ω3 LCPUFA), EPA, in canola seed through genetic engineering. The set of genes was constructed to express the desaturase and elongase fatty acid enzymes of the EPA biosynthetic pathway via seed-directed promotors, which together produce EPA, an ω3 LCPUFA with proven health benefits.

As shown in **Figure 1,** OA is converted to LA by the desaturase Lackl-Δ12D, LA is desaturated to ALA by Picpa-ω3D, ALA to SDA by Micpu-Δ6D, SDA is converted to ETA by Pyrco-Δ6E, and ETA to EPA by Pavsa-Δ5D. All genes are regulated by seed specific promoters. The resulting phenotype is a *B. napus* plant that contains ω3 LC-PUFAs, *i.e.,* EPA and others in the seed oil.

In addition to the desaturase and elongase enzyme genes, the *pat* gene was introduced as a selectable marker to encode PAT which confers tolerance to phosphinothricin and glufosinate ammonium-based herbicides driven by a constitutive promoter. The PAT protein has been introduced in many GM crops that have been consumed without adverse effects to human or animal health.

This is the same pathway and enzymes used in the previously approved DHA canola except that it does not contain the two last enzymes required to produce DHA from EPA. It is anticipated that EPA canola events will produce oil that ranges from 8-12% EPA of total fatty acids.

The fatty acids in EPA canola are identical to those expressed in other deregulated events, such as DHA canola (17-236-01p) and BASF's EPA and DHA (LBFLFK) canola (17-321-01p) as well as EPA naturally occurring in marine sources (i.e., fish, algae). Except for oil profile changes from the ω3 LCPUFA, it is expected that EPA canola is equivalent to conventional canola.

Summary

EPA canola is being developed to produce long chain omega-3 fatty acid with significant amounts of EPA in seed oil through genetic engineering. The set of genes were constructed to express the desaturase and elongase fatty acid enzymes of the EPA biosynthetic pathway, which together produce EPA, and other ω3 LCPUFAs with proven health benefits. This is the same pathway and enzymes used in the previously approved DHA canola event except that it does not contain the final 2 enzymes required to produce DHA from EPA and DPA.

Desaturase and elongases are transmembrane proteins that exist in all types of biological membranes. They play critical roles in regulating the length and degree of unsaturation of fatty acids. Long chain omega-3 fatty acids are essential to human and animal health, EPA canola offers a sustainable source to produce these important nutrients.

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