Author/s	[redacted]
Title (version Nº)	**Intended Markets for Omega 3 DHA Canola, Event NS-B50027-4 Version 2**
Owner	**Nuseed Pty Ltd**
Date	**December 7, 2016**
Project	**Omega 3 canola**
Report Number	**2016-024**
Testing Facility	**Not Applicable**
Test method	**Not Applicable**
GLP	**No GLP**
Confidentiality	**None**

TITLE:
INTENDED MARKETS FOR OMEGA 3 DHA CANOLA, EVENT NS-B50027-4

TABLE OF CONTENTS

ABBREVIATIONS

ALA	α-Linoleic acid, 18:3n
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DHA	Docosahexaenoic acid, 22:6n3
DHA canola	Genetically modified canola, event NS-B50027-4
DPA	Docosapentaenoic acid, 22:5n3
EPA	Eicosapentaenoic acid, 20:5n3
ETA	Eicosatetraenoic acid, 20:4n3
GLA	Gamma linolenic acid, 18:3n6
GRAS	Generally recognized a safe
Ha	Hectares
LA	Linoleic acid, 18:2n6
LC-PUFA	Long chain (≥C20) polyunsaturated fatty acids
Lackl-Δ12D	*Lachancea kluyveri* Δ12-desaturase
Micpu-Δ6D	*Micromonas pusilla* Δ6-desaturase
MMT	Million metric tons
OA	Oleic acid, 18:1n9
Pavsa-Δ4D	*Pavlova salina* Δ4-desaturase
Pavsa-Δ5D	*Pavlova salina* Δ5-desaturase
Picpa-ω3D	*Pichia pastoris* Δ15-/ω3-desaturase
Pyrco-Δ5E	*Pyramimonas cordata* Δ5-elongase
Pyrco-Δ6E	*Pyramimonas cordata* Δ6-elongase
SDA	Stearidonic acid, 18:4n3

I. DESCRIPTION OF THE NATURE AND PURPOSE OF THE INTRODUCED TRAIT

Canola production (also known as rapeseed) has grown rapidly over the past 40 years, rising from the sixth largest oil crop in the world to the second largest. Canola seed is typically not sold to consumers, but is crushed at processing facilities into oil and meal. In 2015/2016, approximately 67 million metric tons (MMT) of oilseed were produced; representing 10% of the world's oilseed production[1]. In 2015, Australia and Canada grew 2.4M Ha and 8.1M Ha, respectively (USDA-FAS, 2016). Although the US share of world production remains small (0.7M Ha in 2015), it is of growing importance to regional economies in the Northern Plains.

Nuseed, in partnership with Commonwealth Scientific and Industrial Research Organisation (CSIRO) has developed a biotechnology derived canola Event NS-B50027-4 (DHA canola) expressing the long chain omega-3 fatty acids Docosahexaenoic acid (DHA), Eicosopentaenoic acid (EPA), and Docosapentaenoic acid (DPA), providing a sustainable source of long chain omega-3 fatty acids to help meet the need for increased dietary intake of these important nutrients.

There is robust scientific literature suggesting the health benefits of long chain omega-3 fatty acids, eicosapentaenoic acid (EPA, 20:5n3) and docosohexaenoic acid (DHA, 22:6n3) (Yurko-Mauro et al. 2015). Although country recommendations differ as to how much EPA and DHA should be consumed daily, it is widely accepted that intake in most populations should be increased (Stark et al. 2016).

There is a finite supply of EPA and DHA derived from wild capture fisheries and rendering of aquaculture fish fed a diet containing wild fish ingredients (Nichols et al. 2010). Additional sources of EPA and DHA from single cell fermentation systems of microalgae and yeast have become available, however at very low volumes. Sustainable and financially accessible technologies are needed to ensure continued access to EPA and DHA sources for existing and expanding markets. Canola combined with biotechnology is a vehicle that can provide a solution to meet the needs of the growing fish oil market.

Nuseed's DHA oil will be extracted from harvested DHA canola grain and contain fatty acids in esterified form comprised of the following:

Oleic acid	C18:1n9	1 – 30%
Palmitic acid	C16:0	2 – 15%

[1] http://www.ers.usda.gov/data-products/oil-crops-yearbook/oil-crops-yearbook/#World Supply and Use of Oilseeds and Oilseed Products

Omega-6 fatty acids comprised of:		
Linolenic acid (LA)	C18:2n6	4 – 35%
Gamma linolenic acid (GLA)	C183n6	0 – 7%
Omega-3 fatty acids comprised of:		
Alpha-linolenic acid (ALA)	C18:3n3	4 – 40%
Docosahexaenoic acid (DHA)	C22:6n3	7 – 35%
and optionally one or more of:		
Eicosopentaenoic acid (EPA)	C20:5n3	7 – 35%
Stearidonic acid (SDA)	C18:4n3	0 – 10%
Docosapentaenoic acid (DPA)	C22:5n3	0 – 10%
Eicosatetraenoic acid (ETA)	C20:4n3	0 – 6%
Total ∑ saturated fatty acids		4 – 20%
Total ∑ monounsaturated fatty acids		4 – 40%
Total ∑ polyunsaturated fatty acids		20 – 75%

The DHA oil will be refined for use as an alternate source of omega 3 fatty acids in existing food ingredient markets for fish oils or established omega 3 markets. Possible target product examples include:

- Dairy products enriched with fish oil: milk (flavoured or plain), cream cheese products, yoghurts, custard desserts and dairy alternatives (soy milk, soy cheese).
- Bread and cereals enriched with fish oil or omega 3: museli, breakfast cereal, cereal bars, white bread, multigrain bread.
- Spreads, condiments and sauces containing omega 3: margarine (or margarine blends), salad dressings, mayonnaise, dips (i.e. hummus).
- Tinned fish in oil: tinned tuna chunks, tinned tuna sandwich filling (plain or flavoured); tinned bean mix.

The inclusion level of DHA oil (or enriched DHA oil) in these foods will depend on the food matrix, oil content of the food and the degree of substitution of the fish oil or omega 3 ingredient. These foods already have omega 3 content or health claims from the inclusion of the fish oil or omega 3 ingredients. Substitution with DHA oil would retain these claims. A target of 30-60 mg DHA/serve would meet the various USA, EU and Australian criteria for content and health related claims in the food. The required amounts of DHA for food content and health related claims differ in jurisdictions around the world.

Worked example:

Hummus dip (20% oil content), 20g serving size, would yield >60mg DHA/serve.

The consumption of DHA in these foods is expected to remain on current trends when DHA oil is used as a fish oil ingredient substitute. Consumption is expected to increase slightly

where DHA oil is used as an omega 3 oil ingredient substitute. Consumption rates of these foods and consumption trends are not expected to change with the introduction of DHA oil, so contemporary dietary modeling programs are considered sufficient.

The market share of DHA oil in the fish oil food ingredient market is likely to be low to begin with, increasing over time and with market acceptance to as high as ~20% after 10 years. Market forces, consumer choices (enriched food vs nutraceutical consumption) and demand for fish oil in all markets (feed, food, nutraceuticals) will determine the rate of adoption and market share of DHA oil in food.

Future food opportunities for DHA oil could present with new technologies of processing or micro-encapsulation/micro-emulsion. These possibilities could include foods like frozen/chilled meals, juice/smoothies or soups. Fish oil is used in infant formula/infant diets but Nuseed are not pursuing this opportunity at this time as we acknowledge additional regulatory approval may be needed in various jurisdictions for these applications.

Refined DHA canola oil can also be used in dietary supplements. Oils used for this application are typically encapsulated into a soft gel form for easier consumption; however flavored liquids, oil-water emulsions and chewable pastels are also becoming more widely available.

In some cases, the natural levels of long chain omega-3 fatty acids may be enriched to increase the relative amounts of EPA and DHA. These products are almost exclusively sold in a soft gel form as dietary supplements or pharmaceutical products; however it is possible to also incorporate them into a liquid formula. Nuseed acknowledges additional regulatory approvals may need to be sought for some of these applications should they be pursued.

Canola and fish oils rich in omega-3 fatty acids are widely used today in animal feed for cattle, swine, poultry, and some aquaculture species. Long chain omega-3 canola oil from DHA canola may be used as an ingredient for animal and aquaculture feed as an alternative to fish oil and other sources of omega-3 feed components. Supplementing animal feeds with omega 3 oils can results in foods like salmon or eggs with increased amounts of omega 3 fatty acids. Nuseed is also considering these opportunities that contribute to the human diet.

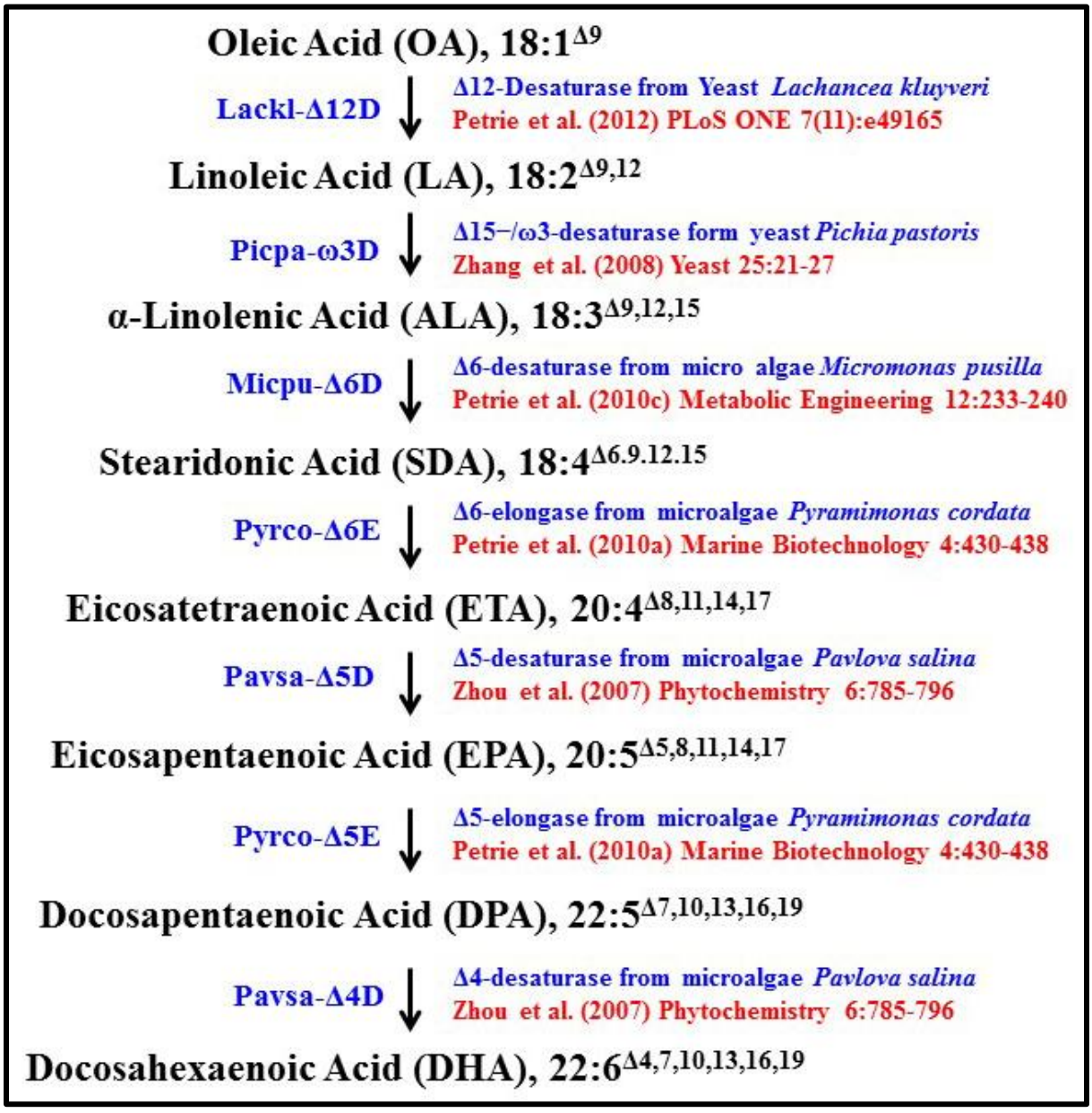
The DHA meal component of DHA canola is compositionally similar to other commodity canola meal and will be used in a manner similar to conventional canola meal, including use in animal feeds.

II. IDENTITY AND INTENDED FUNCTION OF THE GENETIC MODIFICATION

Canola (*B. napus* L.) is one of the most important oilseed crops in the world especially for its oleic acid (OA), linoleic acid (LA) and linolenic acid (ALA). However, it is not a source of LC-PUFAs such as DHA and EPA. About 3.7% DHA and 0.7% EPA were recently achieved in canola seed through the expression of a microalgal polyketide synthase-like PUFA synthase system (Walsh et al. 2016). In collaboration with the CSIRO, Nuseed Pty Ltd has developed a DHA canola that accumulates high concentrations of DHA in seed oil. DHA canola was produced through *Agrobacterium tumefaciens*-mediated transformation of canola cultivar AV Jade with binary vector pJP3416_GA7-modB, which has been specifically designed to convert native OA, LA and ALA to DHA in canola seed, and contains expression cassettes for seven microalgae and yeast genes between the *A. tumefaciens* T-DNA left and right borders.

Seven fatty acid desaturases and elongases were introduced into DHA canola to convert OA to DHA via a biosynthetic pathway (Figure 1). The pathway consists of the *Lachancea kluyveri* Δ12-desaturase, *Pichia pastoris* Δ15-/ω3-desaturase, *Micromonas pusilla* Δ6-desaturase, *Pyramimonas cordata* Δ6-elongase, *Pavlova salina* Δ5-desaturase, *P. cordata* Δ5-elongase and *P. salina* Δ4-desaturase. Based on the sequence similarity and functionality, these seven enzymes can be classified into three groups: (1) yeast acyl-CoA type fatty acid desaturases, Lackl-Δ12D and Picpa-ω3D that introduces a double bond at the Δ12 and Δ15 positions, respectively, (2) algae fatty acid elongases, Pyrco-Δ6E and Pyrco-Δ5E that add a carbon to the carboxyl end of fatty acids, and (3) algae front-end fatty acid desaturases, Micpu-Δ6D, Pavsa-Δ5D and Pavsa-Δ4D that introduce a double bond between an existing double bond and the carboxyl end of fatty acids.

Figure 1. Biosynthetic pathway of long chain ω3 fatty acids described in this study.



III. FISH AND FISH OIL AS A FOOD / FEED SOURCE

Fish is a dietary staple and one of the healthiest foods available. Oily fish have a very high nutritional value, provide an excellent source for protein and vitamin D, and are one of the best sources of omega 3 fatty acids. Nutrition experts recommend 1-2 fish servings per week for a healthy diet, which takes advantage of the benefits of omega 3 fatty acids, specifically DHA and EPA. The benefits of fish oil, DHA and EPA are wide ranging and includes improved heart health, mental health, brain development and function, eye development and health, bone and muscle strength, immune function and may even contribute to healthy body weight (Yurko-Mauro et al. 2015).

Yet reports indicate that only a fraction of the population meet these recommended fish consumption levels. An Australian Health Survey found only about 20% of Australians consumed fish/seafood[2], while vegan and vegetarians are typically severely deficient in DHA (Sanders, 2009). Another source of omega 3 fatty acids is from dietary supplements (typically fish oil), which are usually consumed as encapsulated soft gels, but the fish sourcing is still a problem for vegans. Although fish oil supplements are the most popular natural product in the US, consumption rates remain low, at less than 8% of the adult population (Clarke et al. 2015).

Fish oils and high protein fish meals containing omega 3 fatty acids, are widely used today in animal feed for cattle, swine, poultry, and some aquaculture species. Aquaculture is the major market for fish oil since salmon and other fatty fish require a minimum level of fish oil for efficient growth. The presence of omega 3 oils in the aquaculture feeds, results in deposition of these fatty acids in fish flesh. Supplementing animal feeds with omega 3 oils, today mostly from algae or fish, can results in foods like salmon or eggs with increased amounts of omega 3 fatty acids.

The fish oil supply is fairly consistent at approximately 900,000 metric tonnes of crude oil harvested per year[3] due to fishery quotas and seasonal limits, yet demand in some markets is growing at the expense of supply to others. Fish oil is largely used in the animal feed industry, predominantly aquaculture feeds, while the remainder is split between nutraceuticals and fortified food. It is estimated that about 20-25% of the fish oil market or

[2] ABS. Australian Health Survey: Nutrition First Results- Food and Nutrients, 2011-12,Table 4.1. 2014 [cited 2015 1 October 2015]
[3] http://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/469658/

~210,000 MT, is further refined for use in nutraceutical products. Only about 6,000 MT of fish oil is utilized to fortify foods, half of this is used in infant formulas.[4]

IV. CANOLA AS A FOOD / FEED SOURCE

In the 1940s, rapeseed breeding efforts increased in Canada with a focus on improving agronomic characteristics and oil content. While rapeseed oil was initially utilized as a lubricant for manufacturing purposes, there was a desire to utilize the oil and meal for animal nutrition. However, the relatively high levels of erucic acid (C22:1) in the oil and glucosinolates in the meal limited its use as a nutritional supplement to animal feeds. From the 1960-1990s, traditional breeding programs were initiated and successful in the development of a low erucic acid/low glucosinolate rapeseed, which was named canola.

Canola oil has been used in a wide range of food products over the last 25 years and utilized in nearly all food products requiring an oil source. Canola has a strong history of safe use, which is why canola was chosen as a production vehicle for DHA oil.

Canola oil is also used in many non-food products, such as de-icer for airplanes, suntan oil, biodiesel and soap stock products. Many industries are familiar with handling canola oil.

Canola meal is rarely used in food products; however, there are certain uses of canola protein isolates that have received GRAS status.[5]

Canola meal is an excellent source of protein (36-44%) and fiber for livestock, poultry and fish (OECD, 2011). Since canola meal has about 30% hulls, it is often targeted to ruminants. De-hulled canola meal can be added to monogastric diets. Incorporation rates of 5-30% are routinely used.

4 GOED Proprietary Research 2014, www.goedomega3.com/
5 http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices

V. APPLICATION AND PROPOSED PRODUCT NAME

Given all the targeted commercial applications of DHA oil as an alternate source of omega-3 fatty acids in food and feed, it is anticipated that DHA canola will initially be a low acreage (<2% of the total USA/CAN/AUS canola acreage). In order to derive commercial value from this product, the crop will be grown and processed in an identity-preserved manner in Australia, Canada and/or USA canola growing regions. The oil will be used in feed, food and nutraceutical applications where omega-3 sources are currently being used. The by-product canola meal has been shown to be compositionally comparable to other commodity canola meals and will be used in a manner similar to conventional canola meal. A commercial trade name for the oil or the products containing the oil has not been determined at the time of this submission and will be determined prior to commercial launch of the oil and associated products.

VI. REFERENCES

Clarke TC, Black LI, Stussman BJ, Barnes, PM and Nahin, RL. 2015. Trends in the use of complementary health approaches among adults: United States, 2002-2012. National health statistics reports; no 79. Hyattsville, MD: National Center for Health Statistics.

Nichols PD, Petrie J, Singh S. 2010. Long-chain omega-3 oils - An update on sustainable sources https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3257669/

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. Series of Novel Food and Feeds Nº 24. https://www.oecd.org/env/ehs/biotrack/49343153.pdf

Omega-3 Fatty Acids and Cardiovascular Disease: An Updated Systematic Review. 2016. Agency for Healthcare Research and Quality. https://www.ahrq.gov/research/findings/evidence-based-reports/er223-abstract.html

Sanders, T. 2009. DHA status of vegetarians. *Prostaglandins Leukotrienes Essential Fatty Acids*. 81:137-141. http://dx.doi.org/10.1016/j.plefa.2009.05.013

Stark, KD, Van Elswyk, ME, Higgins, MR, Weatherford, CA, Salem, N. 2016 Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults *Progress in Lipid Res* 63:132-152. http://www.sciencedirect.com/science/article/pii/S0163782715300333

USDA-FAS, 2016. Production, Supply, and Distribution Database

Walsh TA, Bevan SA, Gachotte DJ, Larsen CM, Moskal WA, Owens Merlo PA, Sidorenko LV, Hampton RE, Stoltz V et al. 2016. Canola engineered with a microalgal polyketide synthase-like system produces oil enriched in docosahexaenoic acid. *Nature Biotech* 34: 881-887.

Yurko-Mauro, K, Alexander, DD, Van Elswyk, ME. 2015. Docosahexaenoic acid and adult memory: a systematic review and meta-analysis *PLoS One* doi: 0.1371/journal.pone.0120391