Application to Food Standards Australia New Zealand for the Inclusion of DHA canola, NS-B5ØØ27-4 (OECD ID) in Standard 1.5.2 - Food Derived from Gene Technology

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10 February. 2017

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Abbreviations And Definitions

AA	amino acids
ALA	α-Linolenic acid, $18:3^{\Delta 9,12,15}$ (ω3)
AOF	Australian Oilseeds Federation
CSIRO	Commonwealth Scientific and Industrial Research Organization
DHA	Docosahexaenoic acid, $22:6^{\Delta 4,7,10,13,16,19}$ (ω3)
DHA canola	Genetically modified canola, event NS-B5ØØ27-4
DPA	Docosapentaenoic acid, $22:5^{\Delta 7,10,13,16,19}$ (ω3)
EPA	Eicosapentaenoic acid, $20:5^{\Delta 5,8,11,14,17}$ (ω3)
FSANZ	Food Standards Australia New Zealand
LA	Linoleic acid, $18:2^{\Delta 9,12}$ (ω6)
Lackl-Δ12D	*Lachancea kluyveri* Δ12-desaturase
LC-MS/MS	Liquid chromatography-Tandem Mass Spectrometry
mg	milligram
Micpu-Δ6D	*Micromonas pusilla* Δ6-desaturase
ng	nanogram
OA	Oleic acid, $18:1^{\Delta 9}$
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
ω3 LC-PUFA	Omega-3 long-chain (≥C20) polyunsaturated fatty acids
ORF	Open reading frame
PAT	phosphinothricin N-acetyltransferase
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
PUFA	Polyunsaturated fatty acid
SGF	Simulated gastric fluid

Executive Summary

Nuseed Pty Ltd is a specialised global seed company, committed to Grow Beyond Yield™ by enhancing food and feed performance throughout the value chain. A wholly owned subsidiary of Nufarm Limited (ASX: NUF), Nuseed solves critical nutrition needs with seed-based solutions. Through world-class research, expert teams, collaborative partnerships and a focus on innovation, Nuseed develops top performing hybrids with environmental benefits that drive value through the agrifood chain, solving problems and creating new market opportunities for growers, processors and end-users.

Long-chain omega-3 (LC-ω3) fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have established health benefits. EPA and DHA are primarily sourced from oils extracted from wild-caught fish, which consume algae containing these healthy oils. Wild fish stocks are under pressure due to increasing demand for LC-ω3 fatty acids for aquacultural, nutraceutical and pharmaceutical applications. There is a need for alternative, direct sources of LC-ω3 fatty acids for increased human consumption and demand from aquaculture.

In collaboration with the Commonwealth Scientific and Industrial Research Organization (CSIRO), Nuseed has developed a genetically modified *Brassica napus* (canola) line, DHA canola (OECD ID NS-B5ØØ27-4) that accumulates relevant amount of DHA in seed oil. DHA canola was produced through *Agrobacterium tumefaciens*-mediated transformation of the conventional canola cultivar AV Jade.

The introduced genetic material produces seven enzymes that convert oleic acid in canola to the final product, DHA. These enzymes can be grouped into three classes: two fatty acid desaturases from yeast, two elongases from microalgae, and three front-end desaturases from microalgae. A herbicide-tolerant selectable marker from a bacterium was used in the initial transformation/selection process, but was not used in the breeding process.

The seven introduced fatty acid biosynthesis enzymes that drive the production of DHA using seed-specific promoters were detected in developing seed and mature seed only, at low levels (20-740 ng/mg total protein), while none of the DHA biosynthesis enzymes were detected in the non-seed tissues of DHA canola. No significant homologies to known and putative allergens or toxins were found with the newly expressed proteins. The rapid digestion of the full-length proteins indicates that it is highly unlikely that these proteins will pose any safety concern to human health. The protein safety evaluations of these enzymes in the DHA pathway conclude that there is a reasonable certainty of no harm resulting from DHA canola, including the introduced genes and proteins, in human foods, animal feed or environmentally.

The DHA canola would be grown and processed in the same way that commercial canola is currently grown and processed. The canola meal fraction would be utilised in the same way that commercial canola meal is currently utilised, primarily as animal feed. The oil fraction would be utilised in markets that use LC-ω3 oils, including animal/aquaculture feed, food additives, nutraceuticals and pharmaceuticals.

DHA canola has been released into the environment in Australia under Licence DIR123 from the Office of the Gene Technology Regulator (OGTR) for field trials from 2014, and in Canada from 2016. There have been no reports of harm to human health and safety or the environment resulting from field trials in Australia or overseas. The research data, including molecular, protein, agronomy and composition data, has been compiled and presented in this application, which confirms that the only change in DHA canola was by design to the oil profile.

The DHA canola was identified to have two T-DNA inserts. The phenotypic and molecular stability of the inserts has been tested over five generations and found to be stable. No new open reading frames were created at the insert junctions and there were no significant sequence homologies with any known toxins or allergens.

No unintended changes were observed in the composition or agronomics of DHA canola when compared with its parental variety and other commercial canola varieties.

The purpose of this submission is to make an application to amend the *Australia New Zealand Food Standards Code* Standard 1.5.2 - *Food Produced Using Gene Technology* to allow for the inclusion of Food derived from DHA canola, NS-B5ØØ27-4 in Schedule 26 – *Food Produced using gene technology*. Information is included in this application that cover Part 3.1.1 and 3.5.1 of the application handbook. A submission is being made concurrently to the OGTR for a commercial release licence. Similar applications will be made to relevant agencies overseas as appropriate.

Part 1 General Requirements

1.1 Applicant Details

(a) Applicant's name

Nuseed Pty Ltd

(b) name of contact person

[redacted]

(c) address (street and postal)

Street: 103-105 Pipe Road, Laverton North, Vic, 3026
Postal: PO Box 103, Laverton, Vic, 3028

(d) telephone number

[redacted]

(e) email address

[redacted]

(f) nature of applicant's business

Nuseed Pty Ltd is a specialised global seed company, committed to Grow Beyond Yield™ by enhancing food and feed performance throughout the value chain. A wholly owned subsidiary of Nufarm Limited (ASX: NUF), Nuseed solves critical nutrition needs with seed-based solutions. Through world-class research, expert teams, collaborative partnerships and a focus on innovation, Nuseed develops top performing hybrids with environmental benefits that drive value through the agrifood chain, solving problems and creating new market opportunities for growers, processors and end-users.

(g) details of other individuals, companies or organisations associated with the application.

Nuseed Pty Ltd, in collaboration with the Commonwealth Scientific and Industrial Research Organization (CSIRO), has developed a genetically modified canola line, DHA canola (OECD ID NS-B5ØØ27-4) that accumulates concentrations of omega 3 fatty acids, especially EPA, DPA and with particular emphasis on DHA in canola seed oil. Nuseed Pty Ltd is managing this application for inclusion in Standard 1.5.2 of the *Food Standards Code*.

1.2 Purpose of the Application

The purpose of this submission is to make an application to amend the *Australia New Zealand Food Standards Code* Standard 1.5.2 - *Food Produced Using Gene Technology* to allow for the inclusion of Food derived from DHA canola, NS-B5ØØ27-4 in Schedule 26 – *Food Produced using gene technology*. Information is included in this application that cover Part 3.1.1 and 3.5.1 of the application handbook.

Nuseed has developed a genetically modified canola line, **DHA canola**, which accumulates substantial amounts of DHA in canola seed oil. This event has an OECD identifier of NS-B5ØØ27-4 and is hereafter referred to as DHA canola. This amendment would permit the use of food derived from DHA canola developed by Nuseed.

An application to the Office of the Gene Technology Regulator (OGTR) for a licence for dealings involving intentional release (DIR) of genetically modified plants into the environment - commercial release is being made simultaneously with this application to FSANZ to amend the *Food Standards Code* Standard 1.5.2 - *Food Produced Using Gene Technology*.

1.3 Justification for the Application

(a) The need for the proposed change

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) for human use (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).

Nuseed, in partnership with Commonwealth Scientific and Industrial Research Organisation (CSIRO) has developed a biotechnology derived canola Event NS-B5ØØ27-4 (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.

(b) The advantages of the proposed change over the status quo, taking into account any disadvantages

Omega-3 fatty acids are essential fatty acids: required for human health and obtained primarily from diet. Omega-3 fatty acids are a group of polyunsaturated fatty acids (PUFA) that are important for a number of bodily functions, including muscle activity, blood clotting, digestion, fertility, cell division and growth, and reducing inflammation (Gogus & Smith 2010). Omega-3 PUFAs play a critical role in the development and function of the central nervous system (Bourre 2004; Dyall 2015).

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 humans continue to have expanded access to EPA and DHA and for continued access in animal feed and aquaculture industries.

The demand for EPA and DHA will increase, despite the current, limited source. DHA canola will provide a sustainable alternative to existing sources to meet the increasing market demand for long chain omega-3 fatty acids.

(c) Regulatory Impact Information

<u>Costs and benefits to the consumer</u>
Consumers will benefit from a sustainable source of long chain omega-3 fatty acids which promote various health benefits. The expanded market place will enable greater consumer access to these important fatty acids. Consumers will be able to make an informed choice as a result of labelling requirements and marketing activities.

No additional costs for consumers are foreseen. The greater availability of long chain omega-3 fatty acids may arrest future price increases of this otherwise limited commodity.

Costs and benefits industry and business in general

The benefit to growers and the canola industry would be a higher value crop and access to improved varieties containing the omega-3 trait. The benefits to animal feedlots and aquaculture feed manufacturers would be access to a sustainable and financially viable source of omega-3 fatty acids. The benefits to industry in general include numerous market opportunities where this omega-3 could be utilised when not a limited commodity. Multiple niche opportunities may open for small to medium enterprises, along with larger businesses. Refer to Report No 2016-024.

Some costs foreseen with a market introduction of DHA canola relate to market segregation and identity preservation of the seed and subsequent oil. The canola industry and downstream processors currently manage market segregation and identity preservation for different canola oil profiles, as well as distinguish between genetically modified canola and conventional canola. Therefore any additional costs for segregating DHA canola should be minimal. No additional costs are foreseen in the canola meal fraction as it would be used in exactly the same way as existing canola meal in animal feedlots.

Costs and benefits to government

If the proposed amendment to include food derived from DHA canola is approved by FSANZ, there would be no disruption to the supply chain due to non-compliance with the FSANZ Code for DHA canola or its processed fractions. An approval would also ensure no potential for trade disruption on regulatory grounds and ensure no potential conflict with World Trade Organization responsibilities.

No additional regulatory costs are foreseen. Regulatory agencies already incur costs for monitoring of compliance with various Standards and labelling guidelines. An amendment to the Food standards code for DHA canola would not increase this burden.

Impact on international trade

A market introduction of DHA canola would not be expected to significantly impact the international trade of canola seed, canola oil or products containing canola oil. The DHA canola would be grown and identity preserved to capture the benefit of the oil. DHA canola may even create new trade opportunities for a DHA canola oil, or products containing DHA canola oil. Relevant approvals will be sought in countries where canola seed is traded to ensure compliance in the case of minor cross-contamination or low level presence.

A rejection of the DHA canola application would result in a denial of the benefits of a sustainable and financially accessible source of omega-3 fatty acids and create trade issues in many different commodities when DHA canola is introduced in other world areas.

1.4 Information to support the application

Information has been supplied within this application to meet the general requirements for applications and the guideline requirements of Chapter 3.5.1 of the *FSANZ Application Handbook*. The unpublished Nuseed reports have been listed separately and supplied electronically to FSANZ. The quoted references in this application have also been listed separately with web hyperlinks (where relevant). Where no web hyperlink to a reference was available, the references have been supplied electronically to FSANZ.

1.5 Assessment Procedure

Nuseed Pty Ltd is submitting this application in anticipation that it will fall within the General procedure category.

1.6 Confidential Commercial Information; Other confidential information

There are specific claims within this application for Confidential Commercial Information. There is no confidential information within this particular application document. Eight of the unpublished supporting studies have been redacted to protect confidential commercial information. A separate justification is provided explaining why certain information is confidential. A confidential supplemental attachment is provided containing the redacted information from each of the redacted studies.

No other information is requested to be treated as confidential. It is requested that personal information is not disclosed.

1.7 Exclusive Capturable Commercial Benefit

This application is likely to result in an amendment to the Code that could provide an exclusive benefit or financial gain and therefore Nuseed intends to pay the full cost of processing the application.

1.8 International and Other National Standards or Regulations

There are no relevant international or national standards specifically regarding this application.

The OGTR regulates the environmental release and feed approval of genetically modified plants. An application to the OGTR for a licence for dealings involving intentional release (DIR) of

genetically modified plants into the environment - commercial release is being made simultaneously with this application to FSANZ to amend the *Food Standards Code* Standard 1.5.2 - *Food Produced Using Gene Technology*.

There are comparable regulatory agencies for food/feed approvals and environmental release of genetically modified plants in other developed countries. The submission to FSANZ and OGTR in Australia is the first place of lodgement of the DHA canola data package for regulatory clearance.

Approvals for DHA canola will also be sought from the relevant authorities in the USA and Canada for food, feed and cultivation approval. Deregulation will be sought in other countries as required.

Part 2 Specific Data Requirements for Safety Assessment

Part 2A Technical information on the food produced using gene technology

A.1 Nature and identity of the genetically modified food

(a) a description of the GM organism
from which the new GM food is derived. The description must include the nature and purpose of the genetic modification.

Canola, *Brassica napus*, was genetically modified to produce DHA canola, which contains omega-3 long chain (≥C20) polyunsaturated fatty acids (ω3 LC-PUFA). DHA canola was produced through *Agrobacterium tumefaciens*-mediated transformation of canola cultivar AV Jade with the binary vector pJP3416_GA7-ModB. The vector was specifically designed to convert oleic acid (OA) to docosahexaenoic acid (DHA) in canola seed, and contained expression cassettes of seven microalgae and yeast genes (Micpu-Δ6D, Pyrco-Δ5E, Pavsa-Δ5D, Picpa-ω3D, Pavsa-Δ4D, Lackl-Δ12D and Pyrco-Δ6E) in the DHA biosynthetic pathway, and a herbicide selection marker gene (already assessed by FSANZ) between the *A. tumefaciens* T-DNA left and right borders.

(b) the name, line number and OECD Unique identifier
of each of the new lines or strains of GM organism from which the food is derived

DHA canola, Event number B0050-027, OECD identifier NS-B5ØØ27-4.

(c) the name the food will be marketed under (if known).

There are several market opportunities for omega-3 rich oils, including both food and feed. These are elaborated on in Report 2016-024. DHA canola is a sustainable and financially viable option to supply these markets. No branding or naming conventions for these commercial opportunities have been determined as yet.

A.2 History of use of host and donor organisms

(a) For the donor organism(s) from which the genetic elements are derived:

(i) any known pathogenicity, toxicity or allergenicity of relevance to the food;

(ii) history of use of the organism in the food supply or history of human exposure to the organism through other than intended food use (e.g. as a normal contaminant).

The scientific literature database, PubMed, was searched for evidence that the donor organisms (*Lachancea kluyveri*, *Micromonas pusilla*, *Pavlova salina*, *Pichia pastoris* and *Pyramimonas cordata*) are a likely source of allergy or toxicity. The search did not reveal evidence that these organisms represent any food safety risks. Refer to Report No 2016-017.

Lachancea kluyveri (Lackl-Δ12D)

Yeasts are essential microorganisms in the production of various foods and drinks such as bread, beer, wine and cider. The yeast strain *L. kluyveri,* from which the gene was cloned, is widely used in Emmental, Roquefort, Damietta and Greek cheeses, and fermented milk. The closely related strain *L. lanzarotensis* is naturally present in grape must and contributes to spontaneous alcoholic fermentation during the early phases of wine fermentation, before *Saccharomyces cerevisiae* becomes dominant and completes the process. Refer to Report No 2016-005.

Pichia pastoris (Picpa-ω3D)

Yeasts are essential microorganisms in the production of various foods and drinks such as bread, beer, wine and cider. *P. pastoris* is a species of methylotrophic yeast widely used in recombinant protein techniques (Ahmad et al. 2014). A number of food proteins and enzymes have been expressed in *P. pastoris* (Batt 2014). A number of products obtained by heterologous expression in *P. pastoris* have already found their way to the market (Ahmad et al. 2014), including phytase used as an animal feed additive to cleave plant derived phytate, trypsin used as proteinase in proteomics research, phospholipase C used for degumming of vegetable oils, collagen used for medical research and as a dermal filler, Jetrea® as a drug for treatment of symptomatic vitreomacular adhesion, and recombinant human insulin. Refer to Report No 2016-006.

Micromonas pusilla (Micpu-Δ6D)

Marine microalgae, such as *M. pusilla*, are primary producers in the marine food web.

Pyramimonas cordata (Pyrco-Δ6E; Pyrco-Δ5E)

Marine microalgae, such as *P. cordata*, are primary producers in the marine food web.

Pavlova salina (Pavsa-Δ5D; Pavsa-Δ4D)

Marine microalgae, such as *P. salina*, are primary producers in the marine food web. *P. salina* itself, is one of several microalgae used in mariculture (Brown 1991).

(b) For the host organism into which the genes were transferred:

(i) its history of safe use for food
(ii) the part of the organism typically used as food
(iii) the types of products likely to include the food or food ingredient
(iv) whether special processing is required to render food derived from the organism safe to eat.

Traditional rapeseed (*B. napus* L) was unsuitable as a source of food for either humans or animals due to the presence of two naturally occurring compounds in the seed: erucic acid and glucosinolates. The development of an edible version called 'canola' is described in the OGTR biology document for canola (OGTR 2106) and it has been grown and consumed since the 1980's. The term 'canola' refers to those varieties of *B. napus* L. that meet specific standards on content of erucic acid (below 2 percent) in oil and glucosinolates (total glucosinolates of 30 mmoles/g oil-free air-dry solids) in meal (AOF 2016; Codex 2009). Similar biology documents on canola are available from regulators in other world areas describing the development and history of use of canola.

Canola seed is processed into two main fractions of oil and meal. No special processing is required to render the fractions safe to consume, as the quality composition is specified in the Australian Oilseeds Federation standard (AOF 2016).

Both the oil and the meal are used in food, feed and/or industrial uses. Canola oil is 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 an industrial oil/lubricant as well as a source of biodiesel. Canola meal is widely used as an animal feed for cattle, pig and poultry, with potential for use in aquafeed.

A.3 Nature of genetic modification

(a) a description of the method used to transform the host organism

Canola was transformed via *Agrobacterium*-mediated transformation as described in the OGTR Risk Assessment Reference: Methods of plant genetic modification (OGTR 2012). Further details of the transformation method used on NS-B5ØØ27-4 can be found in Report No 2016-002, Section 4.2 Gene Transformation and Development of DHA Canola.

An antibiotic agent was used to eliminate *Agrobacterium* during *in vitro* selection of the transformed canola plants. The GM plants have been propagated by seed and *Agrobacterium* is not normally transmitted from one generation to the next via seed. The GM plants have been propagated by seed through seven generations. Further molecular analysis and sequencing of the genome of DHA canola, NS-B5ØØ27-4, has found no evidence of the presence of the *Agrobacterium* genome in DHA canola. Further details can be found in Report No 2016-002, Section 4.3 Absence of Vector Backbone Sequence in DHA Canola NS-B5ØØ27-4.

(b) a description of the construct and the transformation vectors used, including:

(i) the size, source and function of all the genetic components including marker genes, regulatory and other elements

(ii) a detailed map of the location and orientation of all the genetic components contained within the construct and vector, including the location of relevant restriction sites.

The plasmid construct pJP3416_GA7-ModB was used to create DHA canola, NS-B5ØØ27-4. A full description of the identity, function, origin, reference and location of each gene from plasmid pJP3416_GA7-ModB is provided in Report No 2016-002, Table 2, and pictorially represented in Figure 3.

(c) A full molecular characterisation of the genetic modification in the new organism, including:

(i) identification of all transferred genetic material and whether it has undergone any rearrangements

(ii) a determination of the number of insertion sites, and the number of copies at each insertion site

(iii) full DNA sequence of each insertion site, including junction regions with the host DNA

(iv) a map depicting the organisation of the inserted genetic material at each insertion site

(v) details of an analysis of the insert and junction regions for the occurrence of any open reading frames (ORFs).

The DHA canola was identified to have two T-DNA inserts. The details are provided in Report No 2016-002, Section 4.4 Presence of Two T-DNA Inserts in DHA Canola.

The molecular analysis of the T-DNA inserts and their flanking canola sequences are provided in Report No 2016-002, Section 4.5, Table 3 and Table 4, and pictorially represented in Figure 4 and Figure 5. The function, source and relevant citation are also provided in Table 3 and Table 4. The sequence of the T-DNA inserts perfectly matched the reference of vector pJP3416_GA7-ModB; thus no amino acid (AA) variations were observed in the protein sequences compared to their references (Report No 2016-002, Section 4.6 and Section 4.7).

Molecular analysis and sequencing of the genome of DHA canola, NS-B5ØØ27-4, has found no evidence of the presence of the *Agrobacterium* genome in the plants. Further details can be found in Report No 2016-002, Section 4.3.

A bioinformatics analysis was conducted on the junctions of the introduced DNA with endogenous canola DNA. No new open reading frames were created during the transformation of canola and there were no significant homologies of the border sequences with toxins or allergens. Refer to Report No 2016-004.

(d) A description of how the line or strain from which food is derived was obtained from the original transformant (i.e. provide a family tree or describe the breeding process) including which generations have been used for each study.

The various generations used for the molecular analysis are listed in Report 2016-002, Table 1. The pedigree including breeding process is provided in Report 2016-002, Figure 1.

(e) Evidence of the stability of the genetic changes, including:

(i) the pattern of inheritance of the transferred gene(s) and the number of generations over which this has been monitored

(ii) the pattern of inheritance and expression of the phenotype over several generations and, where appropriate, across different environments.

The molecular stability of DHA canola, NS-B5ØØ27-4, was tested over five generations from T_2- T_7 and found to be stable. Refer to Report No 2016-002, Section 4.10. Genetic Stability Testing of DHA Canola by Amplification of Four Junction Regions of the two T-DNA Inserts.

The phenotype stability of DHA canola, NS-B5ØØ27-4, was found to be fixed and stable over five generations. Refer to Report 2016-019, Table 11.

DHA canola was also crossed with recurrent parents to produce second generation progeny, which produced the expected Mendelian inheritance of the two different loci (T-DNA inserts). Refer to Report No 2016-019.

The phenotype stability of DHA canola, NS-B5ØØ27-4, was tested by back-crossing with the recurrent parent and producing second generation progeny, which produced the expected Mendelian inheritance of the LC-PUFA trait. The expected and actual percentage LC-PUFA phenotype for the range of BC1F2 (back-crossed second generation) progeny with variable zygosity at either locus is presented in Report No 2016-019, Table 2.

The expression of the phenotype across different environments (glasshouse, field locations) is provided in Report 2016-019, Table 11.

(f) an analysis of the expressed RNA transcripts, where RNA interference has been used.

Not applicable.

Part 2B Characterisation and safety assessment of new substances

PAT protein

The herbicide-tolerant selectable marker was used to identify transformed plants in the initial selection transformation/selection process, but was not used in the breeding process. The safety assessment of phosphinothricin-N-acetyltransferase (PAT) has already been conducted by the FSANZ in several crops. A safety assessment of the PAT protein in transgenic plants has been published (Hérouet et al. 2005). The PAT protein from *S. viridochromogenes* is a member of a well-characterized, safe class of enzymes with a high degree of substrate specificity, and shows significant homology with PAT proteins from other source organisms. A bioinformatics comparison of PAT to known protein toxins and allergens was conducted (refer to Report No 2016-017). The results from the recent bioinformatics searches of the PAT amino acid sequence show and confirm no significant risk of allergy or toxicity. The search results from the AllergenOnline.org database show that the likelihood of allergy or allergic cross-reactivity for the PAT protein is very low. The search results from PubMed and BLASTP on allergens and toxic proteins suggest that the PAT protein has a low risk of food allergenicity or toxicity. No further information is required under the FSANZ guidelines for the PAT protein.

B.1 Characterisation and safety assessment

(a) a full description of the biochemical function and phenotypic effects of all new substances (e.g. a protein or an untranslated RNA) that are expressed in the new GM organism, including their levels and site of accumulation, particularly in edible portions

The seven introduced fatty acid biosynthesis enzymes in the DHA pathway are listed below:

1. Lackl-Δ12D — Δ12-Desaturase from Yeast *Lachancea kluyveri*
   The characterisation, functionality and use in food, food production and in animal feeds of Δ12-Desaturase is discussed in Report No 2016-005.
2. Picpa-ω3D — Δ15-/ω3-Desaturase form Yeast *Pichia pastoris*
   The characterisation, functionality and use in food, food production and in animal feeds of Δ15-/ω3-Desaturase is discussed in Report No 2016-006.
3. Micpu-Δ6D — Δ6-Desaturase from Microalgae *Micromonas pusilla*
   The characterisation, functionality and use in food, food production and in animal feeds of Δ6-Desaturase is discussed in Report No 2016-007.
4. Pyrco-Δ6E — Δ6-Elongase from Microalgae *Pyramimonas cordata*
   The characterisation, functionality and use in food, food production and in animal feeds of Δ6-Elongase is discussed in Report No 2016-008.
5. Pavsa-Δ5D — Δ5-Desaturase from Microalgae *Pavlova salina*
   The characterisation, functionality and use in food, food production and in animal feeds of Δ5-Desaturase is discussed in Report No 2016-009.

6. Pyrco-Δ5E Δ5-Elongase from Microalgae *Pyramimonas cordata*
The characterisation, functionality and use in food, food production and in animal feeds of Δ5-Elongase is discussed in Report No 2016-010.
7. Pavsa-Δ4D Δ4-Desaturase from Microalgae *Pavlova salina*
The characterisation, functionality and use in food, food production and in animal feeds of Δ4-Desaturase is discussed in Report No 2016-011.

A summary of the protein safety evaluations of the seven introduced fatty acid biosynthesis enzymes in the DHA pathway is provided in Report No 2016-016, which concludes that there is a reasonable certainty of no harm resulting from DHA canola, including the introduced genes and proteins, in human foods, animal feed or environmentally.

The presence of all seven LC-PUFA biosynthesis proteins was determined in seven different plant fractions, over five time periods from two locations in 2015. All seven LC-PUFA biosynthesis proteins were then quantified in each fraction by LC-MS/MS. The enzymatic proteins that drive the production of DHA using seed-specific promoters were detected only in developing seed and mature seed at low levels, while none of the DHA biosynthesis proteins were detected in the non-seed tissues of the transgenic canola, irrespective of the sampling time or the tissues tested. Refer to Report No 2016-015.

(b) information about prior history of human consumption of the new substances, if any, or their similarity to substances previously consumed in food.

A discussion of the history of use and their similarity to substances previously consumed as food is provided for the seven fatty acid biosynthesis enzymes in their respective reports, refer Report 2016-005 through 2016-011.

Lackl-Δ12D	Report No 2016-005.
Picpa-ω3D	Report No 2016-006.
Micpu-Δ6D	Report No 2016-007.
Pyrco-Δ6E	Report No 2016-008.
Pavsa-Δ5D	Report No 2016-009.
Pyrco-Δ5E	Report No 2016-010.
Pavsa-Δ4D	Report No 2016-011.

(c) information on whether any new protein has undergone any unexpected post-translational modification in the new host

The translated amino acid sequence of each of the seven fatty acid biosynthesis enzymes in DHA canola was compared to the respective original sequence with no changes found, and the functionality of each enzyme confirmed in Report 2016-005 through 2016-011.

Lackl-Δ12D	Report No 2016-005.

Picpa-ω3D	Report No 2016-006.
Micpu-Δ6D	Report No 2016-007.
Pyrco-Δ6E	Report No 2016-008.
Pavsa-Δ5D	Report No 2016-009.
Pyrco-Δ5E	Report No 2016-010.
Pavsa-Δ4D	Report No 2016-011.

The accumulation of DHA in the oil fraction is also substantial evidence that all of the enzymes are functioning properly and as expected. The composition analysis Report 2016-021 describes the fatty acid profile of DHA canola over eight geographic locations; and the inheritance stability Report 2016-019 describes the stability of the fatty acid profile of DHA canola over five generations.

(d) where any ORFs have been identified (in subparagraph A.3(c)(v) of this Guideline (3.5.1)), bioinformatics analyses to indicate the potential for allergenicity and toxicity of the ORFs.

A bioinformatics analysis was conducted on the junctions of the introduced DNA with endogenous canola DNA. No new open reading frames were created during the transformation of canola and there were no significant homologies of the border sequences with toxins or allergens. Refer to Report No 2016-004.

B.2 New proteins

(a) information on the potential toxicity of any new proteins, including:

(i) a bioinformatic comparison of the amino acid sequence of each of the new proteins to known protein toxins and anti-nutrients (e.g. protease inhibitors, lectins)

(ii) information on the stability of the protein to proteolysis in appropriate gastrointestinal model systems

(iii) an animal toxicity study if the bioinformatic comparison and biochemical studies indicate either a relationship with known protein toxins/anti-nutrients or resistance to proteolysis.

No significant homologies were found from the bioinformatics searches of the newly expressed fatty acid biosynthesis enzymes when compared to known and putative allergens or toxins. Refer to Report No 2016-017.

Based on the sequence similarity and functionality, the seven fatty acid biosynthesis enzymes can be classified into three groups: (1) yeast acyl-CoA type fatty acid desaturases Lackl-Δ12D and Picpa-ω3D that introduce a double bond at the Δ12 and Δ15 positions, respectively; (2) algae

fatty acid elongases Pyrco-Δ6E and Pyrco-Δ5E that add two carbons 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 A representative of each of the three groups of enzymes was analysed to assess the *in vitro* stability in simulated gastric fluid (SGF) comprising the proteolytic enzyme, pepsin, and in combination with a novel pepsin-trypsin assay employing state-of-the-art mass spectrometric approaches to monitor the precise degradation products.

1) Picpa-ω3D: The results of the study demonstrated that greater than 80% was digested within 5 min and greater than 97% of the full-length Picpa-ω3D protein was digested within 60 min of incubation in pepsin when analysed by LC-MS/MS. Refer to Report 2016-012.
2) Pyrco-Δ5E: The results of the study demonstrated that greater than 75% of the Pyrco-Δ5E protein digested within 5 min and full-length protein was rapidly digested within 60 min of incubation in pepsin producing a suite of pepsin products. Refer to Report 2016-013.
3) Pavsa-Δ4D: The results of the study demonstrated that greater than 80% was digested within 10 min and greater than 93% of the full-length Pavsa-Δ4D protein was digested within 60 min of incubation in pepsin when analysed by LC-MS/MS. Refer to Report 2016-014.

The bioinformatics comparison and the biochemical degradation studies do not indicate any relationship with known protein toxins; thus, there is no need for toxicity tests or further evaluation of the seven fatty acid biosynthesis enzymes for potential allergenicity or toxicity.

(b) information on the potential allergenicity of any new proteins, including:

(i) source of the new protein
(ii) a bioinformatics comparison of the amino acid sequence of the novel protein to known allergens
(iii) the new protein's structural properties, including, but not limited to, its susceptibility to enzymatic degradation (e.g. proteolysis), heat and/or acid stability
(iv) specific serum screening where a new protein is derived from a source known to be allergenic or has sequence homology with a known allergen
(v) information on whether the new protein(s) have a role in the elicitation of gluten-sensitive enteropathy, in cases where the introduced genetic material is obtained from wheat, rye, barley, oats, or related cereal grains.

The source and amino acid sequence of the new proteins are presented in Report 2016-005 through 2016-011.

Lackl-Δ12D	Report No 2016-005.
Picpa-ω3D	Report No 2016-006.
Micpu-Δ6D	Report No 2016-007.

Pyrco-Δ6E	Report No 2016-008.
Pavsa-Δ5D	Report No 2016-009.
Pyrco-Δ5E	Report No 2016-010.
Pavsa-Δ4D	Report No 2016-011.

A literature search for evidence that the donor/source organisms were a likely source of allergy or toxicity was conducted and the results presented in Report 2016-017, Table 1. There were no publications found that showed any of the donor organisms are sources of human allergy or toxicity.

No significant homologies were found from the bioinformatics searches of the newly expressed fatty acid biosynthesis enzymes when compared to known and putative allergens or toxins. Refer to Report No 2016-017.

A representative of each of the three groups of fatty acid biosynthesis enzymes was analysed to assess the *in vitro* stability in SGF and a discussion on the thermal stability of trans-membrane enzymes was presented. The fatty acid biosynthesis enzymes were readily digestible in pepsin and/or trypsin. It was also argued that these enzymes will not be present in their native, folded state after processing and hence will have reduced or no activity.

1) Picpa-ω3D: Refer to Report 2016-012.
2) Pyrco-Δ5E: Refer to Report 2016-013.
3) Pavsa-Δ4D: Refer to Report 2016-014.

The safety of the introduced proteins is supported by: the history of safe use of proteins similar to those in DHA canola that have been routinely consumed for many years; their quick digestion in pepsin and/or trypsin; and their lack of similarity to known allergens of toxins using *in silico* analysis. Each protein has been fully characterized and quantitated in DHA canola. The enzymatic proteins that drive the production of DHA using seed-specific promoters were only detected in developing seed and mature seed at low levels (20-740 ng/mg total protein), while none of the DHA biosynthesis pathway enzymes were detected in the non-seed tissues of the transgenic canola, irrespective of the sampling time or the tissues tested.

Agronomic and compositional analyses were conducted across 10 and 8 field trial sites, respectively. The DHA canola values fell within the range of non-transgenic commercial varieties and were comparable to the parental canola variety in both agronomy and composition measurements, aside from the intended changes to the fatty acid pathway (e.g., high DHA).

In conclusion, there is a reasonable certainty of no harm resulting from DHA canola, including the introduced genes and proteins, in human foods, animal feed or environmentally. Report No 2016-016.

Specific serum screening of the new proteins is not required as neither the source of the proteins nor the proteins themselves are indicated as allergenic. No information on gluten-sensitive enteropathy is required as none of the source organisms are from cereal grains.

B.3 Other (non-protein) new substances

The new substances created in DHA canola are long-chain ω-3 fatty acids [by design] from the inclusion of seven fatty acid biosynthesis enzymes that convert native Oleic Acid, $18:1^{\Delta 9}$ (OA), Linoleic Acid, $18:2^{\Delta 9,12}$ (LA) and α-Linolenic Acid, $18:3^{\Delta 9,12,15}$ (ALA) to Docosahexaenoic Acid, $22:6^{\Delta 4,7,10,13,16,19}$ (DHA).

(a) the identity and biological function of the substance

Canola can only produce the short-chain ω-3 fatty acid, α-Linolenic Acid, $18:3^{\Delta 9,12,15}$ (ALA). DHA canola produces the long-chain ω-3 fatty acids, Eicosapentaenoic acid, $20:5^{\Delta 5,8,11,14,17}$ (EPA), Docosapentaenoic acid, $22:5^{\Delta 7,10,13,16,19}$ (DPA) and Docosahexaenoic acid, $22:6^{\Delta 4,7,10,13,16,19}$ (DHA). The oil profile of DHA canola is presented in Report No 2016-021. Long-chain omega-3 fatty acids are well known as beneficial substances with a history of safe use. Refer to Report No 2016-023.

(b) whether the substance has previously been safely consumed in food

Long-chain ω-3 fatty acids are well known as beneficial substances with a history of safe use. Refer to Report No 2016-023. A large variety of fish and fish products, as well as meat, dairy, margarine, and egg products containing DHA are available in grocery stores and supermarkets. Dietary supplements of fish/krill/algae oils containing DHA are readily available from supermarkets and pharmacies.

(c) potential dietary exposure to the substance

DHA is not a new substance and should be a part of everyday dietary intake, therefore a potential dietary exposure assessment to DHA from DHA canola is not warranted. DHA canola will provide an alternate source of DHA for existing markets.

There is robust scientific literature suggesting the health benefits of consuming 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).

ANZ Food Standards Code Schedule 4 – Nutrition, health and related claims contains entries for long chain Omega-3 fatty acids, including DHA. The conditions for permitted general level health claims Part 3 – Other notes EPA and DHA as having the specific health effect of "Contributes to heart health" when part of a "Diet containing 500mg of EPA and DHA per day".

DHA from DHA canola will be offered in various forms and markets, and will become part of the supply chain to meet a growing demand for DHA. Refer to Report 2016-024. Indicative profiles of the oil from DHA canola show the comparative difference in the fatty acid profile [by design] when compared to the parental variety, AV Jade and other reference varieties. Refer to Report 2016-021.

(d) where RNA interference has been used:

(i) the role of any endogenous target gene and any changes to the food as a result of silencing that gene

(ii) the expression levels of the RNA transcript

(iii) the specificity of the RNA interference

Not applicable.

B.4 Novel herbicide metabolites in GM herbicide-tolerant plants

Herbicide tolerance is not claimed in this event as the herbicide-tolerance selection marker was used as an initial selection tool at the transformation stage and not selected for in breeding and development of NS-B5ØØ27-4. A herbicide tolerance use pattern is not being sought from the Australian Pesticides and Veterinary Medicines Authority.

B.5 Compositional analyses

(a) **the levels of relevant key nutrients, toxicants and anti-nutrients in the food produced using gene technology compared with the levels in an appropriate comparator (usually the non-GM counterpart). A statistical analysis of the data must be provided.**

A detailed compositional analysis of DHA canola was conducted in accordance with the revised OECD Consensus Document on Compositional considerations for new varieties of low erucic acid rapeseed (canola; *Brassica napus*) (OECD 2011) and provided in Report 2016-021. DHA canola was compared with its parental variety, AV Jade, and other conventional canola varieties from Australian field trials in 2015. The results were statistically analysed. DHA canola was within the general range of the trial comparators for each analysis and below the maximum levels of erucic acid and glucosinolates that define the quality of canola.

Compositional analysis of grain samples included protein, fat, acid detergent fiber (ADF), neutral detergent fiber (NDF), crude fiber, ash, carbohydrates, fatty acids (FA), amino acids, vitamins, minerals, phytosterols and key anti-nutrients.

Proximate analysis:
No statistically significant differences were identified for acid detergent fiber, crude fiber, neutral detergent fiber and protein. Although statistical differences in the calculated means were identified for ash, carbohydrates and crude fat, the calculated means are very close numerically and the standard deviation and ranges overlap. The means and data ranges fell largely within the reference data ranges for those analytes. Therefore, it is unlikely that these differences indicate any biological significance.

Amino acids and sterols:
No statistically significant differences were identified for ten amino acids. Although statistical differences in the calculated means were identified for eight amino acids, the calculated means are close numerically and the standard deviation and ranges overlap, but this does not raise any nutritional concerns. No statistically significant differences were identified for glucosinolates, except for glucobrassicin. The glucosinolate content of DHA canola was well below the required standard for canola. No statistically significant differences were identified for two phytosterols, although statistical differences were identified for the remainder. The calculated means were close numerically and the standard deviation and ranges overlap. The means and data ranges fell largely within the reference data ranges for those analytes. Therefore, it is unlikely that these differences indicate any biological significance.

Vitamins and minerals:
No statistically significant differences were identified for seven minerals. Although statistical differences in the calculated means were identified for four minerals, the calculated means were close numerically and the standard deviation and ranges overlap. Statistically significant differences were identified for most of the vitamins, but the calculated means were close numerically and the standard deviation and ranges overlap. The means and data ranges fell largely within the reference data ranges for those analytes. Therefore, it is unlikely that these differences indicate any biological significance.

Fatty acids:
Statistically significant differences were expected and found in the fatty acid profile of DHA canola. The erucic acid [C22:1n-9] content of DHA canola was well below the required standard for canola.

(b) information on the range of natural variation for each constituent measured to allow for assessment of biological significance should any statistically significant differences be identified

The composition report (Report 2016-021) includes DHA canola data from five replicates at eight different trial site locations. A comparison was made with data from its parental variety, AV Jade, as well as seven other conventional canola counterparts. These comparators provide a spectrum of natural variation for each constituent measured.

Excluding the fatty acid profile which is different by design, statistically significant differences were identified for some composition analytes. The means and data ranges fell largely within the reference data ranges for those analytes. Therefore, it is unlikely that these differences indicate any biological significance.

An international repository of knowledge on crop composition parameters is also available from the International Life Science Institute Research Foundation.
ILSI, 2016. International Life Sciences Institute Crop Composition Database, Version 6, **www.cropcomposition.org**

(c) the levels of any other constituents that may potentially be influenced by the genetic modification, as a result, for example, of downstream metabolic effects, compared with the levels in an appropriate comparator as well as the range of natural variation.

The intended change in DHA canola is to the oil profile only which has been characterised in Report 2016-021. No other changes to other constituents have been noted or are predicted.

There are no unintended changes predicted in DHA canola because none of the introduced genetic elements are known to affect other metabolic pathways within canola other than the DHA fatty acid pathway. The seven fatty acid enzymes that drive the production of DHA use seed-specific promoters. These enzymes were only detected in developing seed and mature seed at low levels, while none of the DHA pathway enzymes were detected in any other tissues of DHA canola. Refer to Report No 2016-015. This confirms that the seven fatty acid enzymes are only expressed in the seed.

Full molecular characterization of the T-DNA inserts and native canola flanking sequences are presented in Report 2016-002. An open reading frame analysis (Report 2016-004) confirms there are no new open reading frames associated with the T-DNA inserts in DHA canola.

Observations of DHA canola in the glasshouse, in field trials in Australia and overseas did not indicate any unexpected changes. Refer to Report 2016-018.

Part 2C Nutritional impact of GM food

(a) data are required on the anticipated dietary intake of the GM food in relation to the overall diet, together with any information which may indicate a change to the bioavailability of the nutrients from the GM food

DHA is not a new substance and is a part of everyday dietary intake of people, therefore a potential dietary exposure assessment to DHA from DHA canola is not warranted. DHA is an essential fatty acid with established health benefits.

DHA from DHA canola will be offered in various forms and markets, and will become part of the supply chain to meet a growing demand for DHA. Profiles of the oil from DHA canola show the comparative difference in the fatty acid profile [by design] when compared to the parental variety, AV Jade and other reference varieties. Refer to Report 2016-021.

Indicative profiles of the meal (crude and hexane-extracted) from DHA canola show the comparative similarity of various nutritional characteristics when compared to the parental variety, AV Jade. When the mean of the crude and hexane-extracted meals are compared for CMP and GMO, all were within the ranges usually observed in canola meal. Some differences were expected, specifically those reflected in the fatty acid profiles, which were intentionally modified. However, the amount of oil remaining in the meal is significantly reduced, especially after the solvent extraction process. Refer to Report 2016-022.

DHA canola will provide a sustainable alternative source of omega-3 fatty acids for use in food or feed. A large variety of fish and fish products, as well as meat, dairy, margarine, and egg products containing DHA are available in grocery stores and supermarkets. Dietary supplements of fish/krill/algae oils containing DHA are readily available from supermarkets and pharmacies.

The normal dietary intake of omega-3 fatty acids is not expected to change as a result of this product, but with increasing evidence of the health benefits of omega-3 fatty acids a greater awareness is expected. This may result in a general increase in consumption of all sources of omega-3 fatty acids.

(b) where the GM food contains an intended nutritional change, information, such as clinical trial data, must be provided to determine the nutritional impact of the GM food.

Clinical trials with DHA canola oil have not been performed as human consumption was not allowed under the field trial licence and permits from the OGTR. Clinical trials are not warranted to justify the scientifically established health benefits of omega-3 fatty acids, particularly DHA.

There is robust scientific literature suggesting the health benefits of long chain omega-3 fatty acids: EPA, 20:5n3 and DHA, 22:6n3 for human use (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).

No new nutritional or health claims are being sought under this application to FSANZ. The health benefits of omega-3 fatty acids are generally accepted around the world. Certain claims have been established by local authorities:

- in Australia (heart health, FSANZ FSC Schedule 4) https://www.legislation.gov.au/Series/F2015L00474 ;
- in Canada (help reduce/lower triglycerides, Health Canada, Health Claim Assessments) http://www.hc-sc.gc.ca/fn-an/label-etiquet/claims-reclam/assess-evalu/eicosapentaenoic-acid-acide-eicosapentaenoique-eng.php

Part 2D Other information

No acute, chronic or sub-chronic toxicity studies have been conducted with DHA canola seed, DHA canola oil or DHA canola meal. If and when these studies are conducted for deregulation in other jurisdictions, a copy will be provided to FSANZ.

Part 3 Statutory Declaration – Australia

Statutory Declarations Act 1959 [1]

I, Megan Elisabeth Shaw, Australian Regulatory and Compliance Manager, Nuseed Pty Ltd. 103-105 Pipe Road, Laverton North, Vic, 3026, AUSTRALIA

make the following declaration under the *Statutory Declarations Act 1959*:

1. the information provided in this application fully sets out the matters required
2. the information provided in this application is true to the best of my knowledge and belief
3. no information has been withheld that might prejudice this application, to the best of my knowledge and belief

I understand that a person who intentionally makes a false statement in a statutory declaration is guilty of an offence under section 11 of the *Statutory Declarations Act 1959*, and I believe that the statements in this declaration are true in every particular.

[redacted]

[*Signature of person making the declaration*]
Declared at 103-105 Pipe Road, Laverton North, Victoria, 3026, Australia
on 30th of January 2017.

Before me,

[redacted]

30/1/17

103-105 Pipe Road, Laverton North, Victoria, 3026, Australia

STUART JOHN BRADSHAW
103-105 Pipe Road, Laverton North, Vic. 3026
An Australian Legal Practitioner
within the meaning of the
Legal Profession Act 2004.

[1] http://www.comlaw.gov.au/Series/C1959A00052.

[2] A statutory declaration must be made before a prescribed person under the *Statutory Declarations Act 1959*. The list of prescribed persons is available in the Statutory Declarations Regulations 1993 at http://www.comlaw.gov.au/Series/F1996B00198.

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