Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 155

Commercial release of canola genetically modified for omega-3 oil content (DHA canola NS-B5ØØ27-4)

Applicant: Nuseed Pty Ltd

February 2018

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application DIR 155

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application for the intentional, commercial scale release of genetically modified (GM) canola with altered omega-3 oil content in Australia. A Risk Assessment and Risk Management Plan (RARMP) for this application was prepared by the Regulator in accordance with requirements of the *Gene Technology Act 2000* (the Act) and corresponding state and territory legislation, and finalised following consultation with a wide range of experts, agencies and authorities, and the public. The RARMP concludes that this commercial release poses negligible risks to human health and safety and the environment and no specific risk treatment measures are imposed. However, general licence conditions have been imposed to ensure that there is ongoing oversight of the release.

The application

Application number	DIR 155
Applicant	Nuseed Pty Ltd (Nuseed)
Project title	Commercial release of canola genetically modified for omega-3 oil content (DHA canola NS-B5ØØ27-4)[1]
Parent organism	*Brassica napus* L. (canola)
Introduced gene and modified trait	**Seven genes involved in metabolism of long-chain polyunsaturated fatty acids**: • *Lackl-Δ12D* from yeast *Lachancea kluyveri* • *Picpa-ω3D* from yeast *Pichia pastoris* • *Micpu-Δ6D* from microalga *Micromonas pusilla* • *Pyrco-Δ6E* from microalga *Pyramimonas cordata* • *Pavsa-Δ5D* from microalga *Pavlova salina* • *Pyrco-Δ5E* from microalga *Pyramimonas cordata* • *Pavsa-Δ4D* from microalga *Pavlova salina* **One selectable marker gene for glufosinate tolerance**: • *pat* from soil bacterium *Streptomyces viridochromogenes*
Proposed locations	Australia-wide
Primary purpose	Commercial release of the GM canola

Risk assessment

The risk assessment concludes that risks to the health and safety of people or the environment from the proposed dealings, either in the short or long term, are negligible.

The risk assessment process considers how the genetic modification and activities conducted with the GMO might lead to harm to people or the environment. Risks are characterised in relation to both the seriousness and likelihood of harm, taking into account information in the application, relevant previous

[1] The title of the application submitted by Nuseed is "Commercial release of *Brassica napus* genetically modified for omega-3 oil content, DHA canola".

approvals, current scientific knowledge and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP. Both the short and long term risks are considered.

Credible pathways to potential harm that were considered included: toxic and allergenic properties of the GM canola; potential for increased weediness of the GM canola relative to unmodified plants; and vertical transfer of the introduced genetic material to other sexually compatible plants.

The principal reasons for the conclusion of negligible risks are: the introduced proteins and the end products are not considered toxic or allergenic to people or toxic to other desirable organisms; proteins similar to the introduced proteins are widespread in the environment; the GM canola has been grown in field trials in Australia since 2014 without adverse or unexpected effects; the GM canola and its progeny can be controlled using standard weed management; and the GM canola has limited capacity to establish in undisturbed natural habitats. In addition, food made from the GM canola has been assessed and approved by Food Standards Australia New Zealand as safe for human consumption.

Risk management

The risk management plan concludes that risks from the proposed dealings can be managed so as to protect people and the environment by imposing general conditions to ensure that there is ongoing oversight of the release.

Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan evaluates and treats identified risks and considers general risk management measures. The risk management plan is given effect through licence conditions.

As the level of risk is assessed as negligible, specific risk treatment is not required. However, the Regulator has imposed licence conditions regarding post-release review (PRR) to ensure that there is ongoing oversight of the release and to allow the collection of information to verify the findings of the RARMP. The licence also contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements, which include an obligation to report any unintended effects.

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Abbreviations

ALA	α-linolenic acid
ARA	Arachidonic acid
APVMA	Australian Pesticides and Veterinary Medicines Authority
CCI	Confidential Commercial Information under section 185 of the Gene Technology Act 2000
cm	Centimetres
DIR	Dealings involving Intentional Release
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DPA	Docosapentaenoic acid
DPAn-6	Omega-6 docosapentaenoic acid
EPA	Eicosapentaenoic acid
DTA	Docosatetraenoic acid
ETA	Eicosatetraenoic acid
FSANZ	Food Standards Australia New Zealand
GLA	γ-linolenic acid
GM	Genetically modified
GMO	Genetically modified organism
GRDC	Grains Research and Development Corporation
ha	Hectare
HGT	Horizontal gene transfer
km	Kilometre(s)
LA	Linoleic acid
LC-ω3-PUFA	Long chain omega-3 polyunsaturated fatty acid
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
LGA	Local government area
LOD	Limit of detection
m	Metre(s)
µg	Microgram(s)
mg	Milligram(s)
NHMRC	National Health and Medical Research Council
ng	Nanogram(s)
OA	Oleic acid
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
PRR	Post release review
PUFA	Polyunsaturated fatty acid
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
SDA	Stearidonic acid
the Act	The Gene Technology Act 2000

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.

2. The Act in conjunction with the Gene Technology Regulations 2001 (the Regulations), an inter-governmental agreement and corresponding legislation in States and Territories, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.

3. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

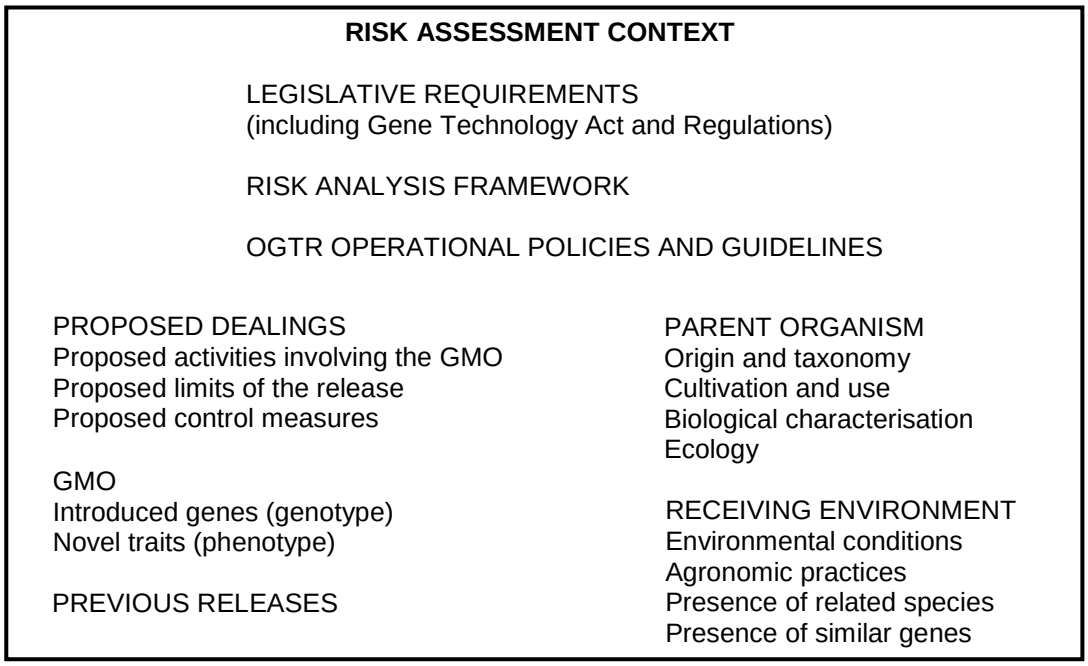


Figure 1 Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and who must be consulted, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that inform the decisions on licence applications. In addition, the Regulations outline further matters the Regulator must consider when preparing a RARMP.

5. Since this application is for commercial purposes, it cannot be considered as a limited and controlled release application under section 50A of the Act. Therefore, under section 50(3) of the Act, the Regulator was required to seek advice from prescribed experts, agencies and authorities on matters relevant to the preparation of the RARMP. This first round of consultation included the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, all Australian local councils and the Minister for the Environment. A summary of issues contained in submissions received is given in Appendix A.

6. Section 52 of the Act requires the Regulator, in a second round of consultation, to seek comment on the RARMP from the experts, agencies and authorities outlined above, as well as the public. Advice from the prescribed experts, agencies and authorities in the second round of consultation, and how it was taken into account, is summarised in Appendix B. One public submission was received and the consideration is summarised in Appendix C.

7. The Risk Analysis Framework (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the legislative requirements. Additionally, there are a number of operational policies and guidelines developed by the Office of the Gene Technology Regulator (OGTR) that are relevant to DIR licences. These documents are available from the OGTR website.

8. Any dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration and the Department of Agriculture and Water Resources. These dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

Section 3 The proposed release

9. Nuseed Pty Ltd (Nuseed) proposes commercial cultivation of GM canola that has been genetically modified for omega-3 oil content, and more specifically for the production of docosahexaenoic acid (DHA). The GM canola line proposed for release is DHA canola with the OECD unique identifier NS-B5ØØ27-4, which is also referred to as Elite event B0050-027.

10. The applicant is seeking approval for the release to occur Australia-wide, subject to any moratoria imposed by States and Territories for marketing purposes. The GM canola could be grown in all commercial canola growing areas, and products derived from the GM plants would enter general commerce, including use in human food and animal feed.

11. The dealings involved in the proposed intentional release are:

(a) breeding the GMO with other canola cultivars

(b) propagating the GMO

(c) using the GMO in the course of manufacture of a thing that is not the GMO

(d) growing the GMO

(e) importing the GMO

(f) transporting the GMO

(g) disposing of the GMO

and the possession, supply or use of the GMO for the purposes of, or in the course of, any of the above.

Section 4 The parent organism

12. The parent organism is *Brassica napus* L., which is commonly known as canola, rapeseed or oilseed rape. Canola is exotic to Australia and is grown as an agricultural crop mainly in Western Australia, New South Wales, Victoria and South Australia. It is Australia's third largest broad acre crop (ABARES, 2017). Canola is primarily grown for its seed oil, which is used as cooking oil and for other food and industrial applications. The seed meal which remains after oil extraction is used as animal feed (OECD, 2011). Information on the weediness of the parent organism is summarised below and information on the use of the parent organism in agriculture is summarised in Section 6 (the receiving environment). More detailed information can be found in *The Biology of* Brassica napus *L. (canola) and* Brassica juncea *(L.) Czern. & Coss. (Indian mustard)* (OGTR, 2017a), which was produced to inform the risk assessment

process for licence applications involving GM canola plants and is available from the OGTR Biology Documents page.

13. The Standards Australia *National Post-Border Weed Risk Management Protocol* rates the weed risk potential of plants according to properties that strongly correlate with weediness for each relevant land use (Standards Australia et al., 2006). These properties relate to the plants' potential to cause harm (impact), to its invasiveness (spread and persistence) and to its potential distribution (scale). The weed risk potential of volunteer canola has been assessed using methodology based on the *National Post-Border Weed Risk Management Protocol* (see Appendix 1, OGTR, 2017a). Please note that, because canola has been grown in Australia over several decades, its actual rather than potential distribution is addressed.

4.1 Potential to cause harm

14. In summary, as a volunteer (rather than as a crop), non-GM canola is considered to exhibit the following potential to cause harm:

- low potential to negatively affect the health of animals and/or people
- limited ability to reduce the establishment or yield of desired plants
- low ability to reduce the quality of products or services obtained from land uses
- limited potential to act as a reservoir for plant pests, pathogens or diseases.

15. *B. napus* seeds contain two natural toxicants: erucic acid and glucosinolates. Erucic acid is found in the oil, and animal feeding studies have shown that traditional rapeseed oil with high levels of erucic acid can have detrimental health effects. Glucosinolates are found in the seed meal, which is used exclusively as livestock feed. The products of glucosinolate hydrolysis have negative effects on animal production (OECD, 2011).

16. The term canola refers to varieties of *B. napus* that contain less than 2% erucic acid in the oil and less than 30 µmoles/g of glucosinolates in the seed meal, so are considered suitable for human and animal consumption (OECD, 2011). The Australian canola crop grown in 2014 contained on average less than 0.1% erucic acid in the oil and approximately 12 µmoles/g of glucosinolates in the meal (Seberry et al., 2015).

4.2 Invasiveness

17. With regard to invasiveness, non-GM canola has:

- the ability to reproduce by seed, but not by vegetative means
- short time to seeding
- high annual seed production
- low ability to establish amongst existing plants
- low tolerance to average weed management practices
- low ability to undergo long distance spread by natural means
- high potential for long distance spread by people from cropping areas and low potential for long distance spread by people from intensive land uses such as roadsides.

4.3 Actual distribution

18. In Australian agricultural settings, volunteer canola is considered to be a major problem warranting control (Groves et al., 2003). Canola volunteers requiring weed management are likely to be found in fields for up to three years after growing a canola crop (Salisbury, 2002; AOF, 2014) but the seedbank declines rapidly (Baker and Preston, 2008). Canola volunteers produce allelopathic compounds that reduce germination of other crops, in addition to directly competing with crop plants (Gulden et al., 2008; Asaduzzaman et al., 2014).

19. Feral canola plants are often observed growing on roadsides or railway easements in Australia; in the case of roadside canola typically within 5 m from the edge of the road (Agrisearch, 2001; Norton, 2003). Roadside canola populations are usually transient, and are thought to be reliant on re-supply of seed through spillages (Baker and Preston, 2004; Crawley and Brown, 2004; Gulden et al., 2008). Due to its primary colonising nature, canola can take advantage of disturbed habitats such as roadside verges, field margins, wastelands and along railway lines. However, canola is a poor competitor with weed species and will be displaced unless the habitats are disturbed on a regular basis (Salisbury, 2002; OECD, 2012).

20. Canola is not considered a significant weed in natural undisturbed habitats in Australia (Dignam, 2001; Groves et al., 2003). Canola seed burial in an undisturbed habitat is likely very low, which might have limited the potential for feral canola populations to persist through secondary dormancy (Busi and Powles, 2016).

Section 5 The GM canola

5.1 Introduction to the GMO

21. DHA canola contains seven introduced genes sourced from yeast and marine microalgae, which are involved in the metabolism of long-chain polyunsaturated fatty acids (with 20 or more carbons). It also contains a selectable marker gene.

22. The genes introduced into DHA canola are listed in Table 1.

Table 1 Introduced genes in DHA canola

Gene	Encoded protein	Source organism	Intended function*
Lackl-Δ12D	Δ12-desaturase	Yeast (*Lachancea kluyveri*)	Convert OA to LA
Picpa-ω3D	Δ15-/ω3-desaturase	Yeast (*Pichia pastoris*)	Convert LA to ALA
Micpu-Δ6D	Δ6-desaturase	Microalga (*Micromonas pusilla*)	Convert ALA to SDA
Pyrco-Δ6E	Δ6-elongase	Microalga (*Pyramimonas cordata*)	Convert SDA to ETA
Pavsa-Δ5D	Δ5-desaturase	Microalga (*Pavlova salina*)	Convert ETA to EPA
Pyrco-Δ5E	Δ5-elongase	Microalga (*Pyramimonas cordata*)	Convert EPA to DPA
Pavsa-Δ4D	Δ4-desaturase	Microalga (*Pavlova salina*)	Convert DPA to DHA
pat	Phosphinothricin acetyl transferase	Soil bacterium (*Streptomyces viridochromogenes*)	Selectable marker (tolerance to glufosinate herbicides)

*****ALA, α-linolenic acid ($18:3^{\Delta 9,12,15}$); DHA, docosahexaenoic acid ($22:6^{\Delta 4,7,10,13,16,19}$); DPA, docosapentaenoic acid ($22:5^{\Delta 7,10,13,16,19}$); EPA, eicosapentaenoic acid ($20:5^{\Delta 5,8,11,14,17}$); ETA, eicosatetraenoic acid ($20:4^{\Delta 8,11,14,17}$); LA, linoleic acid ($18:2^{\Delta 9,12}$); OA, oleic acid ($18:1^{\Delta 9}$); SDA, stearidonic acid ($18:4^{\Delta 6,9,12,15}$)

23. Short regulatory sequences that control expression of the introduced genes are also present in DHA canola. These regulatory elements are listed in Table 2.

Table 2 Introduced regulatory elements in DHA canola

Element	Function	Source
PRO Linus-Cnl1	Seed-specific Promoter	*Conlinin 1* gene from *Linum usitatissimum* (flax)
PRO Linus-Cnl2	Seed-specific Promoter	*Conlinin 2* gene from *L. usitatissimum*
PRO Arath-FAE1	Seed-specific Promoter	*FAE1* gene from *Arabidopsis thaliana*
PRO Brana-FP1	Seed-specific Promoter	*napA* gene from *Brassica napus* (canola)
CaMV 35S	Constitutive Promoter	35S gene from Cauliflower mosaic virus
TER Linus-Cnl1	Terminator	*Conlinin 1* gene from *L. usitatissimum*
TER Linus-Cnl2	Terminator	*Conlinin 2* gene from *L. usitatissimum*
TER Glyma-Lectin	Terminator	Lectin gene from *Glycine max* (soybean)
TER Agrtu-NOS	Terminator	Nopaline synthase gene from *Agrobacterium tumefaciens*
MAR Nicta-RB7	Matrix attachment region (MAR) for increasing gene expression	*Nicotiana tabacum* (tobacco)
TMV 5'-untranslated leader sequence	Enhancer	Tobacco mosaic virus

24. In DHA canola, all the introduced genes coding for fatty acid desaturases and elongases are controlled by seed specific promoters. This results in accumulation of DHA in the GM canola seed, as well as other changes in fatty acid composition compared to non-GM canola. The fatty acid profile of DHA canola has been declared confidential commercial information (CCI) under section 185 of the Act. The confidential information is made available to the prescribed experts and agencies that were consulted during preparation of the RARMP and are consulted on the RARMP for this application. DHA is not naturally produced in non-GM canola.

5.2 The introduced genes, their encoded proteins and associated effects

5.2.1 The yeast and microalgal genes, their proteins and end products

25. DHA canola contains seven introduced genes sourced from yeast and marine microalgae (Table 1). These genes encode fatty acid desaturases[2] and fatty acid elongases[3] that form a novel long chain polyunsaturated omega-3 fatty acid (LC-ω3-PUFA) biosynthesis pathway to convert the native monounsaturated omega-9 fatty acid, OA, to the final LC-ω3-PUFA product, DHA, in the seed (Figure 2). These seven genes were all synthesised and codon optimised for expression in higher plants.

26. DHA canola contains two genes sourced from yeast (*Lackl-Δ12D* and *Picpa-ω3D*), which code for acyl-CoA-type fatty acid desaturases.

27. The *Lackl-Δ12D* gene (GenBank accession BAD08375, originally known as *Sk-FAD2*) is derived from the budding yeast *Lachancea kluyveri* (also known as *Saccharomyces kluyveri*), which codes for a Δ12-desaturase (Δ12D) (Watanabe et al., 2004). Expression of the introduced *Lackl-Δ12D* gene in a *fad2* mutant of *Arabidopsis*, which is deficient in its endogenous Δ12-desaturase activity, showed high activity in Δ12-desaturation (Petrie et al., 2012). The intended purpose of this gene in DHA canola is to create a double bond at the 12th position on OA (18:1$^{\Delta 9}$) to produce LA (18:2$^{\Delta 9,12}$).

[2] A fatty acid desaturase removes two hydrogen atoms from a specific position within a fatty acid to create a carbon-carbon double bond.

[3] A fatty acid elongase adds two carbon atoms to a fatty acid, making the aliphatic chain longer.

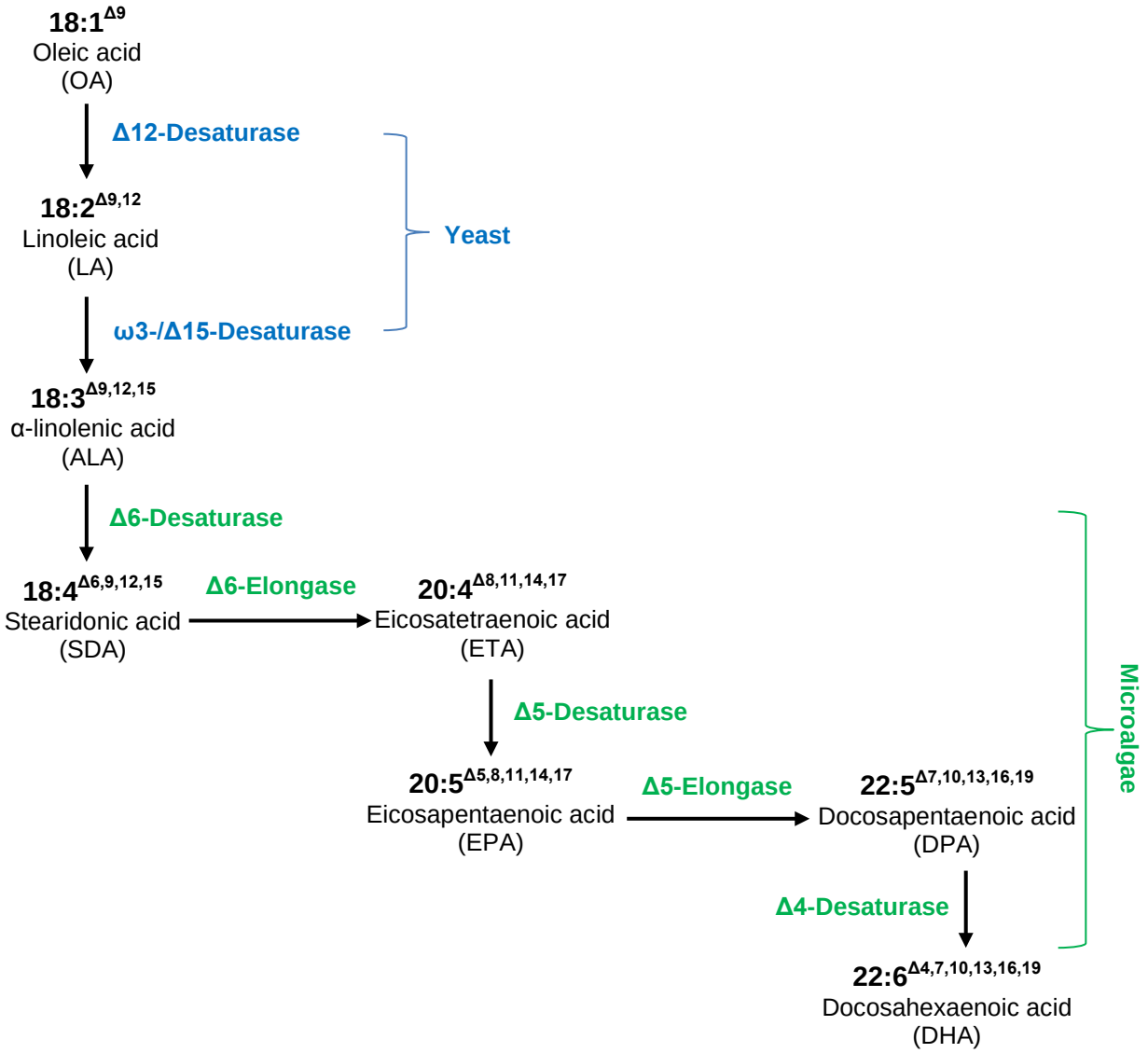


Figure 2. The introduced LC-ω3-PUFA pathway in DHA canola, sourced from yeast and marine microalgae

28. The *Picpa-ω3D* gene (GenBank accession EF116884, originally known as *Pp-fad3*) is derived from the methylotrophic yeast *Pichia pastoris* and codes for a Δ15-desaturase (also called ω3-desaturase) (Petrie et al., 2012). This desaturase has been characterized as having a broad omega-3 specificity (Zhang et al., 2008). The intended purpose of this gene in DHA canola is to catalyse the conversion of LA ($18:2^{\Delta 9,12}$) to ALA ($18:3^{\Delta 9,12,15}$) by creating a double bond at the 15^{th} carbon position.

29. The short chain fatty acids OA, LA and ALA are naturally produced in non-GM canola. The addition of the two yeast genes is designed to increase the production of ALA in DHA canola seed and ultimately enhance the DHA production.

30. DHA canola also contains five genes from marine microalgae, which are named here as *Micpu-Δ6D*, *Pyrco-Δ6E*, *Pavsa-Δ5D*, *Pyrco-Δ5E* and *Pavsa-Δ4D*. Among these genes, *Micpu-Δ6D*, *Pavsa-Δ5D* and *Pavsa-Δ4D* encode front-end fatty acid desaturases [i.e. desaturases that introduce a double bond between an existing double bond and the carboxyl end (Δ) of fatty acids]. *Pyrco-Δ6E* and *Pyrco-Δ5E* encode fatty acid elongases.

31. The *Micpu-Δ6D* gene is derived from the marine microalga *Micromonas pusilla* and encodes a Δ6-desaturase (Δ6D). This Δ6D catalyses the conversion of either LA ($18:2^{\Delta 9,12}$) to γ-linolenic acid (GLA,

18:3$^{\Delta6,9,12}$) or ALA (18:3$^{\Delta9,12,15}$) to SDA (18:4$^{\Delta6,9,12,15}$) by creating a double bond at the 6th carbon position from the carboxyl terminus. In GM *Arabidopsis*, the Δ6D encoded by the introduced *Micpu-Δ6D* gene has been shown to have preference for the omega-3 substrate ALA (Petrie et al., 2010c). Similar Δ6 desaturases from other microalgae, such as *Ostreococcus tauri*, also showed similar substrate preference (Domergue et al., 2005; Ruiz-Lopez et al., 2013). The critical difference between the Δ6-desaturases from higher plants and microalgae is that the former ones are phospholipid-dependent, while the latter ones are acyl-CoA-dependent (Petrie et al., 2010c; Ruiz-Lopez et al., 2013). By introducing the acyl-CoA-dependent Δ6 desaturase genes into higher plants, the omega-3 pathway is promoted, resulting in less accumulation of intermediates from the omega-6 pathway associated with the expression of phospholipid-dependent Δ6 desaturases (Abbadi et al., 2004; Ruiz-Lopez et al., 2012).

32. The *Pyrco-Δ6E* gene is obtained from the marine microalga *Pyramimonas cordata* and codes for a Δ6-elongase (Δ6E) (Petrie et al., 2010a). In DHA canola, Δ6E elongates SDA (18:4$^{\Delta6,9,12,15}$) to ETA (20:4$^{\Delta8,11,14,17}$) by adding two carbons to the carboxyl end. The *Pavsa-Δ5D* gene is derived from another marine microalgal species*, Pavlova salina* (Zhou et al., 2007). This gene encodes a Δ5-desaturase (Δ5D), which converts ETA to EPA (20:5$^{\Delta5,8,11,14,17}$) by creating a double bond at the 5th carbon position. The *Pyrco-Δ5E* gene, also derived from *P. cordata*, encodes a Δ5-elongase (Δ5E), which elongates EPA (20:5$^{\Delta5,8,11,14,17}$) to DPA (22:5$^{\Delta7,10,13,16,19}$) (Petrie et al., 2010a). The *Pavsa-Δ4D* gene from *P. salina* encodes a Δ4-desaturase (Δ4D) (Petrie et al., 2010b). Δ4D generates a double bond at the 4th carbon position on DPA (22:5$^{\Delta7,10,13,16,19}$) to produce DHA (22:6$^{\Delta4,7,10,13,16,19}$).

33. A combination of these five microalgae genes has been shown to result in an efficient conversion of plant fatty acid substrates to DHA in the leaf of *Nicotiana benthamiana* (Petrie and Singh, 2011). The LC-ω3-PUFA pathway comprised of the seven genes described above has been tested in *Arabidopsis thaliana*, which resulted in the accumulation of up to 15% DHA in the seed oil (Petrie et al., 2012).

34. ALA is a key substrate in the omega-3 LC-PUFA pathway and is naturally produced in non-GM canola. The inclusion of the two yeast desaturase genes in DHA canola is to increase the production of ALA, which can thus contribute to increasing the synthesis and accumulation of LC-ω3-PUFAs such as EPA, DPA and eventually DHA. With the enhancement of the omega-3 pathway, the levels of omega-6 LC-PUFAs such as arachidonic acid (ARA), docosatetraenoic acid (DTA) and omega-6 docosapentaenoic acid (DPA*n*-6) would be kept very low (Petrie and Singh, 2011). As a result, DHA canola accumulates a high proportion of DHA relative to other fatty acids in the seed oil.

5.2.2 The pat gene and its protein

35. DHA canola also contains a *pat* gene from the soil bacterium *Streptomyces viridochromogenes*. The *pat* gene codes for the enzyme phosphinothricin acetyl transferase (PAT), which confers tolerance to herbicides containing glufosinate ammonium, and was used in the laboratory to select GM plants during the early stage of development. A number of GM cotton and canola lines containing the *pat* or *bar* gene encoding the PAT protein, have been approved for commercial release both in Australia (DIR 021/2003, DIR 062/2005, DIR 091, DIR 108, DIR 138 and DIR 143) and overseas. Among them, licences DIR 021/2003, DIR 108 and DIR 138 were issued for commercial production of GM canola varieties expressing the PAT protein. No adverse effects on humans, animals or the environment have been reported from any such releases (CERA, 2011; OGTR, 2017b).

5.2.3 Toxicity and allergenicity of the yeast and microalgal proteins

36. As described above, the seven introduced genes in the LC-ω3-PUFA pathway were sourced from yeast and marine microalgae. Although they were synthesised and codon optimised for improved expression in higher plants (information provided by the applicant), their encoded proteins (desaturases and elongases) are exactly the same as their original counterparts in yeast or microalgae.

37. The American Type Culture Collection (ATCC) classifies the budding yeast *L. kluyveri* and the methylotrophic yeast *P. pastoris* as Biosafety Level 1, for organisms that are not known to cause disease in healthy adults (ATCC, 2016). *P. pastoris* has been used as a heterologous expression system for

production of a range of pharmaceutical products (Ahmad et al., 2014). *P. pastoris* dried yeast is permitted to be used as an additive to the feed formulation of broiler chickens as a source of protein (CFR – Code of Federal Regulations Title 21).

38. Among the estimated 5000 species of microalgae throughout the world, about 80 produce phycotoxins that can cause toxicity to humans through fish and shellfish that consume them (Smayda, 1997; Van Dolah, 2000; Brett, 2003; Hallegraeff, 2003). However, the microalgae *P. salina*, *M. pusilla* and *P. cordata* are all recorded as non-toxic in the Australian National Algae Culture Collection, CSIRO.

39. Bioinformatic analysis may assist in the assessment process by predicting, on a purely theoretical basis, the toxic or allergenic potential of a protein. The results of such analyses are not definitive and should be used only to identify those proteins requiring more rigorous testing.

40. A report prepared by Goodman (2016) from the Food Allergy Research and Resource Program (FARRP) evaluated the sequence similarity of the introduced proteins in DHA canola against proteins with known or putative toxicity or allergenicity. Sequence similarity search against the Entrez Protein dataset from the National Center for Biotechnology Information using the BLASTP program (Altschul et al., 1997) (Version 2.6.0) found that none of the introduced proteins share sequence similarities with known protein toxins or allergens based on E scores and identity matches. The degrees of similarity between each introduced protein product and allergenic proteins listed in the AllergenOnline database (Version 16, updated January 2016) from the FARRP, was further evaluated using the FASTA3 sequence alignment program (Pearson, 2000). None of the introduced proteins had immunological relevant similarities with any of the known allergens in the database. Our consideration of the bioinformatics analysis conducted by Goodman (2016) supports the view that it is not necessary to conduct further assessments of the introduced proteins by serum IgE binding studies for potential cross-reactivity (Goodman, 2008).

41. As discussed in Section 5.2.1 and based on sequence similarity and functionality, the seven introduced proteins can be divided into three groups: two yeast acyl CoA-type fatty acid desaturases (Lackl-Δ12D and Picpa-ω3D), two microalgal fatty acid elongases (Pyrco-Δ6E and Pyrco Δ5E) and three microalgal front-end desaturases (Micpu-Δ6D**,** Pavsa-Δ5D and Pavsa-Δ4D). One representative protein from each of the groups was selected (Picpa-ω3D, Pyrco Δ5E, and Pavsa-Δ4D) for *In vitro* digestibility tests using fusion proteins derived from heterologous expression systems (*E. coli* strain C41 or insect cell line *Sf9*) (Colgrave et al., 2016a, b; Colgrave et al., 2016c; MacIntosh et al., 2017). The proteins were subjected to digestion in simulated gastric fluids comprising pepsin or a combination of pepsin and trypsin. The results showed that these selected proteins were readily digestible in pepsin and/or trypsin. FSANZ has analysed the protease cleavage sites in the amino acid sequences of all seven introduced proteins using the PeptideCutter28 tool and concluded that all the proteins were potentially as susceptible to digestion as the vast majority of dietary proteins (FSANZ, 2017b).

5.2.4 Toxicity and allergenicity of DHA

42. In addition to OA, LA and ALA that are naturally produced in non-GM canola seed, DHA canola seed contains high levels of DHA as the end product of the introduced novel LC-ω3-PUFA pathway, as well as lower levels of some intermediates in the pathway (Figure 1, also see Section 5.5.3). More detailed results for fatty acid composition have been declared confidential commercial information (CCI) under section 185 of the Act. The confidential information is made available to the prescribed experts and agencies that were consulted during preparation of the RARMP and are consulted on the RARMP for this application.

43. The pathway intermediates, including SDA, ETA, EPA and DPA see Figure 2), are novel ω3-PUFAs in DHA canola. All these ω3-PUFAs are constituents of fish oils and safe for human consumption, and have been shown to have beneficial effects on human health (Whelan, 2009; Byelashov et al., 2015; Dyall, 2015). However, since DHA is the predominant novel LC-ω3-PUFA in the DHA canola seed, further discussions will be focused on the effects of DHA only.

44. A four-year clinical trial showed no identifiable risks associated with long-term consumption of DHA by patients with a genetic eye condition (Wheaton et al., 2003). A recent study also showed that DHA-enriched canola oil can improve high-density lipoprotein (HDL) cholesterol, triglycerides, and blood pressure and therefore reduce coronary heart disease risk compared with other oils varying in unsaturated fatty acid composition (Jones et al., 2014). DHA from breast milk was determined to be safe up to at least 315mg daily in infants aged 1-6 months (Lien, 2009).

45. Although no toxicity or allergenicity has been associated with the consumption of DHA, some concerns over potential adverse effects from elevated intakes (>3g/day) of highly unsaturated fatty acid (eg EPA and DHA) have been raised. These potential effects include adversely influencing glycemic control (particularly with type 2 diabetics), raising low-density lipoprotein (LDL) cholesterol levels and enhancing susceptibility of LDL oxidation (Whelan and Rust, 2006). However, a later report showed that daily intake of DHA from fish oil up to 7.5 g/day by adults did not result in any consistent adverse responses in lipid levels, *in vivo* oxidation parameters and glycemic control (Lien, 2009).

46. FSANZ has assessed the safety of DHA rich oils from various marine microalgae, including *Ulkenia* sp. and *Schizochytrium* sp., and approved their use as food (FSANZ, 2005) and infant formula products (FSANZ, 2017a).

47. Animal feeding studies have been conducted to assess the safety of DHA. A 90-day genotoxicity and subchronic toxicity study reported that rats fed with up to 2g/kg body weight/day of DHA rich algal or fish oils showed no treatment-related effects in clinical observations, food and water consumption, mortality, gross pathology and histopathology, except for increased body weight and liver weight (Blum et al., 2007). Another 90-d toxicology evaluation showed that male rats fed daily with DHA-rich algal oil up to 3679 mg/kg body weight/day had no observed adverse effects, including general condition and appearance, neurobehavioral endpoints, growth, feed and water intake, ophthalmoscopic examinations, routine hematology and clinical chemistry parameters, urinalysis, or necropsy findings (Schmitt et al., 2012).

48. A recent study on American tree swallows (*Tachycineta bicolor*) showed that chicks fed with diets high in EPA and DHA (1.82% ALA, 3.74% EPA and 3.44% DHA) grew faster and had greater immunocompetence than chicks fed with diets high in ALA and low in EPA and DHA (6.25% ALA, 1.47% EPA and 1.42% DHA), but no significant differences were found in head-bill or tarsus growth rates (Twining et al., 2016). This indicated that EPA and DHA can improve the overall fitness and performance of tree swallow especially where food quantity was limiting.

49. Another recent feeding study on the effects of EPA and DHA on the cabbage white butterfly (*Pieris rapae*) revealed that diets containing EPA and DHA did not affect developmental phenology, larval or pupal weight, food consumption, nor cause larval mortality when the maximum combined amount of EPA and DHA was set at 2.4 µg/mg diet dry weight (0.8 µg/mg of DHA). However, the increasing amounts of EPA and DHA in larval diets (0.7, 1.4, 1.8 and 2.4 µg/mg diet dry weight) resulted in progressively heavier adults, with smaller wings and a higher frequency of wing deformities (Hixson et al., 2016).

5.3 The regulatory sequences

50. All the regulatory elements used in DHA canola are listed in Table 2.

51. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct gene transcription. Seed-specific promoters were used to control the expression of all seven genes involved in the LC-ω3-PUFA pathway. This includes promoters PRO Linus-Cnl1 and PRO Linus-Cnl2 of the *Conlinin 1* and *Conlinin 2* genes from flax (Chaudhary et al., 2001; Truksa et al., 2003), PRO Brana-FP1 of the *nap A* gene from canola (Stalberg et al., 1993) and PRO Arath-FAE1 of the *FAE1* gene from thale cress (*A. thaliana*) (Rossak et al., 2001). Expression of the selectable marker gene *pat* is driven by a 35S promoter from the cauliflower mosaic virus (CaMV) (Kay et al., 1987), which leads to constitutive expression of the *pat* gene in all plant tissues.

52. Also required for gene expression in plants is a messenger RNA termination region (terminator), including a polyadenylation signal. The terminators used in DHA canola include TER Linus-Cnl1 and TER Linus-Cnl2 of the *Conlinin 1* and *Conlinin 2* genes from flax (Chaudhary et al., 2001; Truksa et al., 2003), TER Glyma-Lectin of the *Le1* gene from soybean (Cho et al., 1995) and TER Agrtu-NOS of the nopaline synthase gene from the soil bacterium *Agrobacterium tumefaciens* (Bevan, 1984).

53. Other short regulatory sequences were also used in DHA canola for enhancing gene expression. This includes a matrix attachment region (MAR) from tobacco (Hall et al., 1991; Halweg et al., 2005) and a 5'-untranslated leader sequence from Tobacco mosaic virus (TMV) (Gallie et al., 1987).

54. Although *A. tumefaciens*, CaMV and TMV are plant pathogens, and tobacco produces toxins and carcinogens, the regulatory sequences comprise a small part of their total genome, and in themselves have no pathogenic, toxic or carcinogenic properties. With the exception of tobacco, which is no longer grown commercially in Australia, all the source organisms for the introduced regulatory sequences are present in the Australian environment and thus humans and other organisms would commonly encounter them.

5.4 Method of genetic modification

55. DHA canola was generated by *Agrobacterium*-mediated plant transformation. Information about this transformation method can be found in the document *Methods of plant genetic modification* available from the Risk Assessment References page on the OGTR website.

56. The parental canola variety used for genetic modification was AV Jade, which is not currently grown commercially in Australia. AV Jade was transformed with *A. tumefaciens* strain AGL1 containing a binary vector pJP3416_GA7-ModB. The T-DNA of pJP3416_GA7-ModB harbours all seven genes for LC-ω3-PUFA production plus the selectable marker gene *pat* (Figure 3).

57. After transformation, plant cells were cultured in the presence of phosphinothricin (PPT, synonym of glufosinate ammonium), and subsequently shoots were grown in growth medium supplemented with PPT, to select for PPT tolerance conferred by the introduced *pat* gene. Regenerated plants were evaluated and DHA canola was identified as the line with the preferred molecular and phenotypic characteristics.

58. The GM canola event may be introgressed into elite non-GM canola varieties.

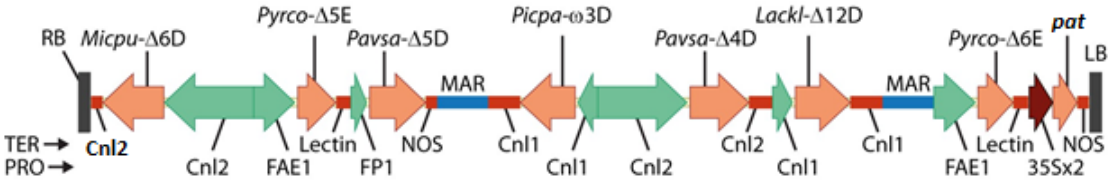


Figure 3 Schematic diagram of the T-DNA region in the binary vector pJP3416_GA7-ModB (modified from Petrie et al., 2014)

Abbreviations: LB, T-DNA left border; RB, T-DNA right border; PRO, promoter; TER, terminator. Refer to Table 1 for all gene names and Table 2 for the identities of the regulatory elements.

5.5 Characterisation of the GMO

5.5.1 Molecular characterisation

59. DHA canola was characterised with vector-targeted sequencing, whole-genome sequencing and PCR-amplicon sequencing. Data provided by the applicant indicate that DHA canola contains two T-DNA inserts in its genome and both T-DNA inserts are required for achieving the current level of DHA in the seed (Tang et al., 2016). The details regarding the exact locations and nature of the T-DNA inserts in the

genome of DHA canola have been declared CCI and are made available to the prescribed experts and agencies that were consulted during preparation of the RARMP and are consulted on the RARMP for this application.

60. Whole genome sequencing confirmed that no vector backbone sequence from pJP3416_GA7-ModB, nor any genomic DNA from *A. tumefaciens* were present in the GM canola (Tang et al., 2016).

61. DHA canola has been advanced to the seventh generation through single-seed descent. The T-DNA inserts were stably inherited from T_0 to T_7 generation, and the seed consistently produced the expected amounts of DHA in T2 to T7 seeds. Segregation analysis of the two T-DNA inserts found segregation ratios consistent with the expected Mendelian inheritance (Tang et al., 2016).

5.5.2 Expression of the introduced proteins

62. The applicant measured protein expression levels of all introduced proteins in tissues of DHA canola and its parental variety AV Jade, collected from two field trial sites at Horsham, Victoria in 2015 (Colgrave et al., 2016d). Protein quantification was conducted by multiple reaction monitoring (MRM) (Gillette and Carr, 2013), following proteolytic digestion of the target protein by trypsin and liquid chromatography.

63. The MRM quantification confirmed that none of the introduced LC- ω3-PUFA pathway enzymes were detected in 250 µg of total protein extracts from the control AV Jade in all growth stages from 5-true-leaf to mature seed, for samples collected from both field trial sites. However, all the seven enzymes were detected in DHA canola developing and/or mature seeds but not in other tested tissues including root, flower and whole plant. This is as expected because the expression of all these genes is controlled by seed-specific promoters. The expression level of each of the enzymes in developing and mature seeds was determined and is shown in Table 3.

Table 3 Expression levels of introduced proteins in DHA canola grown in Australia during 2015

Protein	Developing seed (ng/mg total extracted protein)		Mature seed (ng/mg total extracted protein)	
	Site 1	Site 2	Site 1	Site 2
Lackl-Δ12D	244.2 + 6.8	222.3 + 72.0	212.4 + 43.2	265.4 + 42.0
Picpa-ω3D	167.7 + 24.8	168.1 + 71.0	224.1 + 90.3	263.3 + 26.3
Micpu-Δ6D	87.9 + 15.2	136.1 + 30.3	45.3 + 4.0	42.8 + 7.9
Pyrco-Δ6E	26.1 + 1.8	29.7 + 6.5	ND	ND
Pavsa-Δ5D	63.4 + 16.4	65.8 + 31.7	62.3 + 15.2	75.0 + 20.0
Pyrco-Δ5E	ND	ND	20.0 + 12.1	28.0 + 4.9
Pavsa-Δ4D	480.5 + 146.2	438.2 + 310.2	739.5 + 201.5	724.7 + 154.7

ND, not detected

The applicant used the *pat* gene in DHA canola as a selectable marker in the laboratory and does not intend to use herbicide tolerance as a modified trait for DHA canola production or breeding purposes. The expression levels of the PAT protein in various tissues of DHA canola were measured using the same MRM method as for other introduced proteins. Using this method, expression of the PAT protein was reported to be below the limit of detection in tested tissues including root, flower, whole plant and developing/mature seed. The low level expression of the PAT protein is also confirmed by Western blot analysis (Colgrave et al., 2016d).

5.5.3 Phenotypic and agronomic characterisation

GM canola phenotype

64. The agronomic performance of DHA canola was assessed in field trials in Australia from 2014 to 2016 and in Canada during 2016. The applicant submitted field trial data obtained from the 2015-16 trials in Australia across eight sites in western Victoria and the 2016 trials in Canada at two sites in Alberta and Saskatchewan (Leonforte and Connelly, 2016). The field trials included six different transformation events (including DHA canola) producing DHA in the seed, the parental canola variety AV Jade as control and seven agronomically diverse non-GM canola varieties widely grown in Australia as comparators.

65. The phenotypic characteristics measured represent characteristics that influence reproduction, crop survival and potential weediness. They were plant emergence, plant vigour, flowering time (50%), flowering end, flowering duration, plant height at maturity, seed shattering at maturity, lodging at maturity, plant survival at maturity, grain yield, grain moisture and seed oil percentage. Blackleg disease symptoms were also measured.

66. With the exception of plant lodging at maturity, statistically significant variations ($F<0.001$) were found in all other phenotypic characteristics listed above among DHA canola, the parent AV Jade and the comparator varieties grown both in Australia and Canada. Statistically significant variations ($F<0.001$) between sites were also found for some phenotypic characteristics including plant emergence, flowering time (50%), flowering end, flowering duration, plant height at maturity, plant survival at maturity and grain yield, indicating the effects of environmental differences across experimental sites for these traits. However, all the variations for DHA canola were within the range obtained from the comparator varieties except for seed oil percentage, which was significantly lower than the comparator varieties across all experiments.

67. The combined-site analysis of data from the Australian sites also confirmed that the means of all tested agronomic traits of DHA canola, except for the seed oil percentage, fall within the ranges of comparator variety means. The difference in the seed oil percentage is expected due to the change of oil profile in DHA canola.

68. Statistically significant difference was observed for blackleg disease leaf symptoms among DHA canola, AV Jade and the comparator varieties at both Canadian sites. DHA canola had a higher level of disease than AV Jade and the maximum value from the comparator varieties at one site but within the range for the comparator varieties at the other site. However, the symptoms observed at all Australian sites were all at very low levels, possibly due to low disease pressure. Symptoms associated with cankering and stem breakage were not observed across all Australian and Canadian sites, indicating that DHA canola is similar to non-GM canola in terms of blackleg resistance.

69. In summary, the differences in agronomic performance between DHA canola and non-GM canola are within the range of variation between lines tested and locations, indicating that DHA canola is agronomically similar to non-GM canola varieties.

Compositional analysis

70. The applicant also provided data for compositional analysis of DHA canola seed (T_4 generation) harvested from eight field trial sites in Australia in 2015, in comparison to the parental variety AV Jade and seven reference non-GM commercial canola varieties (Stadler et al., 2017a). Compositional analysis was conducted in accordance with the revised OECD consensus document on compositional considerations for canola (OECD, 2011). The analytes include proximates (ash, carbohydrate, crude fat, fibre and protein), amino acids and sterols, fatty acids, minerals, vitamins and anti-nutrients (glucosinolates, phytic acid, sinapine and tannins).

71. For most analytes, there was no statistically significant difference between DHA canola and the control AV Jade seed. However, statistically significant reductions ($P < 0.05$) were found for some

analytes, with DHA canola seed having reduced levels of ash, crude fat, brassicasterol, campesterol, proline and calcium. Statistically significant increases ($P < 0.05$) were found for some analytes, with DHA canola seed having increased levels of aspartic acid, glycine, lysine, threonine, tyrosine, delta-5-avenasterol, sitosterol, total phytosterols, iron, potassium, alpha- and total tocopherol (vitamin E), niacin (vitamin B3) and thiamin (vitamin B1) (see Table 4 to Table 8). Nonetheless, the levels for each of these analytes were within the tolerance ranges calculated for the reference non-GM canola varieties. Therefore, it is unlikely that these differences indicate any biological significance.

72. Statistical analyses were not conducted for the following analytes: gluconapoleiferin, stigmasterol, delta-7-stigmastenol, sitostanol, soluble tannins, *p*-coumaric acid, neoglucobrassicin, molybdenum and chloride mineral as their values were below the limit of quantitation (LOQ). Although some other analytes showed significant differences, including cholesterol, clerosterol, delta-7-avenasterol, 24-methylene cholesterol, delta-5,24-stigmastadienol, copper, manganese, sodium, zinc, beta-tocopherol, biotin, pantothenic acid (vitamin B5), pyridoxine (vitamin B6), riboflavin (vitamin B2) and vitamin K, their values were very low and close to LOQ. Therefore, it is unlikely that there is biological relevance associated with the statistical differences.

Table 4 Proximate levels (%DW) that are significantly different (P<0.05) between the seed of DHA canola and the parental variety AV Jade

Analyte	DHA canola	AV Jade	Reference range
Ash	3.8±0.4	3.7±0.5	2.7-4.5
Carbohydrates	35.4±2.0	33.0±2.3	27.3-42.3
Crude fat	30.5±2.7	33.2±2.9	25.5-42.1

Table 5 Amino acid levels (%DW) that are significantly different (P<0.05) between the seed of DHA canola and AV Jade

Analyte	DHA canola	AV Jade	Reference range
Alanine	1.268±0.046	1.239±0.049	0.999-1.340
Aspartic acid	2.282±0.097	2.164±0.106	1.680-2.420
Glycine	1.584±0.061	1.519±0.062	1.240-1.660
Lysine	1.948±0.129	1.890±0.107	1.490-2.140
Methionine	0.623±0.027	0.611±0.023	0.490-0.670
Proline	1.865±0.091	1.925±0.086	1.460-2.050
Threonine	1.318±0.045	1.280±0.044	1.040-1.360
Tyrosine	0.817±0.029	0.789±0.035	0.644-0.839

Table 6 Mineral levels (%DW) that are significantly different (P<0.05) between the seed of DHA canola and AV Jade

Analyte	DHA canola	AV Jade	Reference range
Calcium	0.312±0.048	0.356±0.061	0.204-0.488
Iron	0.007±0.001	0.006±0.001	0.004-0.008
Potassium	0.782±0.082	0.666±0.093	0.532-0.915
Zinc	0.005±0.001	0.004±0.001	0.003-0.006

Table 7 Vitamin levels that are significantly different (P<0.05) between the seed of DHA canola and AV Jade

Analyte	DHA canola	AV Jade	Reference range
Biotin	0.07±0.00	0.05±0.00	0.05-0.09
Choline	276.05±23.33	262.73±21.59	195.37-381.31
Niacin (Vitamin B3)	15.14±1.91	9.66±0.96	8.41-16.80
Pantothenic acid (Vitamin B5)	0.56±0.11	0.46±0.10	0.20-0.82
Pyridoxine (Vitamin B6)	0.85±0.10	0.54±0.06	0.44-0.98
Riboflavin (Vitamin B2)	0.35±0.03	0.32±0.06	0.20-0.58
Thiamin (Vitamin B1)	1.48±0.23	1.29±0.20	0.19-2.27
Vitamin K1	0.05±0.01	0.05±0.01	0.03-0.07
α-tocopherol (Vitamin E)	15.69±5.78	11.94±6.61	10.90-31.30
β-tocopherol (Vitamin E)	0.12±0.05	0.08±0.10	LOD-0.65
Total Tocopherols (Vitamin E)	38.88±5.90	33.68±7.20	24.50-96.90

Table 8 Sterol levels (µg/g) that are significantly different (P<0.05) between the seed of DHA canola and AV Jade

Analyte	DHA canola	AV Jade	Reference range
Brassicasterol	0.052±0.004	0.112±0.005	0.045-0.170
Campesterol	0.385±0.018	0.287±0.010	0.226-0.397
Clerosterol	0.006±0.000	0.006±0.000	0.004-0.006
δ_5_avenasterol	0.044±0.008	0.036±0.006	0.008-0.037
Sitosterol	0.579±0.036	0.551±0.028	0.346-0.580
Stigmasterol	0.000±0.001	0.003±0.000	LOD-0.005
24-methylene cholesterol	0.011±0.004	0.013±0.005	0.003-0.020
δ_5 24-stigmastadienol	0.009±0.001	0.007±0.001	0.003-0.009
Total phytosterols	1.106±0.061	1.025±0.040	0.702-1.097

73. Among the anti-nutrients, no statistically significant differences were identified for phytic acid and the following glucosinolates: epiprogoitrin, glucoalyssin, glucobrassicanapin, gluconapin, gluconasturtin, progoitrin, 4-hydroxyglucobrassicin. Although statistical difference was identified for increased glucobrassicin and reduced sinapine, again their levels were within the tolerance ranges calculated for the reference non-GM canola varieties and therefore it is unlikely to indicate any biological significance.

74. The applicant also provided a report for compositional analysis of processed seed meal from DHA canola and the parental variety AV Jade using seed samples harvested from two Australian trial sites in 2015 (Stadler et al., 2017b). The same standard parameters as measured for the whole seed were included in this analysis, comparing both crude meal and hexane extracted meal. When the mean of the crude and hexane-extracted meals are compared for DHA canola and AV Jade, most values are within 10% of each other for most analytes (except for the fatty acid profiles). While some differences were above this 10% level, all were within the ranges usually observed in canola meal.

75. Because DHA canola contains the introduced novel LC- ω3-PUFA pathway, it is not surprising that many of the fatty acids in the seed are different from non-GM canola. As an introduced trait, DHA canola accumulates significant amount of DHA in seed oil. The applicant has applied to protect the information in relation to fatty acid profile of DHA canola seed. This information has been declared CCI and is made available to the prescribed experts and agencies that were consulted during preparation of the RARMP and are consulted on the RARMP for this application. As a general indication, DHA canola seed contains a significantly higher amount of polyunsaturated fatty acids (PUFAs) than its parental variety AV Jade and other comparator non-GM canola varieties.

Section 6 The receiving environment

76. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. Relevant information about the receiving environment includes abiotic and biotic interactions of the crop with the environment where the release would occur; agronomic practices for the crop; presence of plants that are sexually compatible with the GMO; and background presence of the gene(s) used in the genetic modification (OGTR, 2013).

77. The applicant has proposed to release DHA canola in all commercial canola growing areas, Australia-wide. Therefore, for this licence application, it is considered that the receiving environment is all of Australia but in particular agricultural areas that are suitable to cultivate canola. Canola growing areas are mainly in the Australian winter cereal belt of NSW, Victoria, South Australia, and Western Australia. Small quantities of canola are grown in Southern Queensland and Tasmania (OGTR, 2017a). The actual locations, number of sites and area of land used in the proposed release would depend on factors such as field conditions, grower demand and seed availability.

6.1 Relevant agronomic practices

78. In Australia, canola is commonly grown in rotation with wheat as the following crop. Canola is usually grown as a winter annual crop, with planting occurring in April or May and harvest in early summer. Small areas of canola are also sown in late spring/early summer and harvested in early autumn in cool regions with high water availability. Canola has higher requirements for nitrogen, phosphorous and sulphur than most other crops so fertiliser application is important. Canola is harvested either by windrowing (swathing) or less commonly by direct harvesting. Windrowing involves cutting the crop and placing it in rows to dry. After 1-2 weeks, when most of the seed has matured and the moisture content is under 9%, the windrow is picked up by the harvester. Standard cultivation practices for canola are discussed in more detail in *The Biology of* Brassica napus *L. (canola) and* Brassica juncea *(L.) Czern. & Coss. (Indian mustard)* (OGTR, 2017a) and *Canola best practice management guide for south-eastern Australia* (GRDC, 2009).

79. It is anticipated that agronomic practices for the cultivation of DHA canola will not differ from the current standard industry practices. However, an Identity Preservation System will be used in the harvesting/processing practices to ensure that product integrity and/or purity in relation to the oil profile is maintained along the supply chain from seed selection, sowing, grain production to delivery (AOF, 2004).

80. The applicant has noted that the glufosinate herbicide tolerance of DHA canola would not be used in DHA canola production. Glufosinate herbicides are not usually used for weed control in canola production in Australia (GRDC, 2009). Routine weed control in agricultural and non-agricultural areas will be achieved predominantly by herbicides such as glyphosate and 2,4-D (information provided by the applicant), as DHA canola showed similar sensitivity to these herbicides when compared to its parental variety AV Jade (Leonforte, 2016).

6.2 Relevant abiotic factors

81. The geographical distribution of commercial canola cultivation in Australia is limited by a number of abiotic factors, the most important being water availability. Canola is generally grown as a winter crop in dominant winter rainfall environments that receive more than 400 mm rainfall per year. It can be grown in lower-rainfall zones as an opportunistic crop when there is good subsoil moisture, or at low plant population densities to reduce water requirements. Germination of seed will only occur if there is sufficient soil moisture, and drought stress after anthesis can significantly reduce yield due to abortion of seed and reduced pod numbers. Canola is also sensitive to waterlogging, which restricts root development (Walton et al., 1999; GRDC, 2009).

82. Other abiotic stresses that can reduce canola yields include frost, particularly during early pod development, and heat stress (GRDC, 2009). Additional information regarding factors relating to the

growth and distribution of commercial canola in Australia is discussed in the reference document, *The Biology of* Brassica napus *L. (canola) and* Brassica juncea *(L.) Czern. & Coss. (Indian mustard)* (OGTR, 2017a).

6.3 Relevant biotic factors

83. A number of diseases have the potential to significantly reduce the yield of canola. Blackleg disease caused by the fungal pathogen *Leptosphaeria maculans* is the most serious disease affecting commercial canola production in Australia. Blackleg is managed by choosing varieties with high blackleg resistance ratings and by planting canola at least 500 m from the previous year's stubble, which carries blackleg spores. Other damaging diseases of canola include stem rot caused by the fungus *Sclerotinia sclerotiorum* and damping-off caused mainly by the fungus *Rhizoctonia solani* (Howlett et al., 1999; GRDC, 2009).

84. Canola is most susceptible to insect pests during establishment of the crop, at which time earth mites, lucerne flea and false wireworms cause the greatest damage. Damage can also be caused by aphids, native budworm and Rutherglen bug from flowering to podding (Miles and McDonald, 1999; GRDC, 2009).

85. Canola is highly susceptible to weed competition during the early stages of growth. The most problematic weeds include grassy weeds, such as annual ryegrass, vulpia and wild oat, volunteer cereals, and weeds from the *Brassicaceae* family, which can also reduce product quality through seed contamination. The most detrimental *Brassicaceae* weeds are wild radish (*Raphanus raphinastrum*), Indian hedgemustard (*Sisymbrium orientale*), shepherd's purse (*Capsella bursa pastoris*), wild turnip (*Brassica tournefortii*), turnip weed (*Rapistrum rugosum*), charlock (*Sinapis arvensis*), musk weed (*Myagrum perfoliatum*) and Buchan weed (*Hirschfeldia incana*) (Sutherland, 1999; GRDC, 2009).

6.4 Presence of related plants in the receiving environment

86. Canola is predominantly self-pollinating but outcrossing can be mediated by insects, wind or physical contact. The rate of outcrossing between plants averages around 30%. Outcrossing frequencies between adjacent fields are highest in the first 10 m of the recipient fields, and rates decline with distance (Hüsken and Dietz-Pfeilstetter, 2007). Under Australian conditions, a large scale study found that outcrossing rates between neighbouring commercial canola fields were less than 0.1% averaged over whole fields (Rieger et al., 2002).

87. Canola is widely grown as a commercial crop in Australia. Most of the canola crop is herbicide tolerant with one of three different herbicide tolerance traits. In 2015, the Australia canola crop comprised of approximately 60% non-GM triazine tolerant (TT), 15% non-GM imidazolinone tolerant (Clearfield®), 20% GM Roundup Ready® and 5% non-herbicide tolerant canola varieties (OGTR, 2017a). The Clearfield® trait is also available in Juncea canola (*Brassica juncea* or Indian mustard) (DPI NSW, 2013).

88. In addition, TruFlex™ Roundup Ready® canola, a newer variant of Roundup Ready® canola, has been approved for commercial release by the Regulator (DIR 127), but has not yet entered commercial production in Australia. GM Optimum™ GLY Canola, which is also tolerant to glyphosate herbicides but contains a different glyphosate-resistant gene than that used by Roundup Ready® canola, was approved for commercial release by the Regulator in 2016 (DIR 139). Therefore, this canola may be commercially grown in Australia in the near future. GM InVigor® canola, which has tolerance to glufosinate herbicides, was approved for commercial release by the Regulator either alone (DIR 021/2003) or combined with Roundup Ready® canola (DIR 108). However, these canola varieties have only been grown on a limited scale for breeding work and not yet entered commercial production in Australia.

89. Canola can cross with other *B. napus* subspecies including forage rape and vegetables such as swedes if there is synchronicity of flowering. Brassica vegetables are generally harvested prior to

flowering unless they are grown for seed production, in which case precautions would usually be taken to avoid crossing with oilseed canola (OGTR, 2017a).

90. Canola can spontaneously cross with the related crop species *B. juncea* (Indian mustard or Juncea canola) and *B. rapa* (including turnips) (Warwick et al., 2003; Liu et al., 2010), and there is one report of field crosses with the crop species *B. oleracea* (including cabbage, cauliflower and broccoli) (Ford et al., 2006). Juncea canola is grown in Australia as a broad-acre crop similar to canola, though at much smaller scale, and typically in low rainfall regions that are marginally suitable for canola (GRDC, 2009). Horticultural crops that are variants or subspecies of *B. napus*, *B. juncea*, *B. rapa* or *B. oleracea* are also commercially grown in Australia.

91. Under open pollination conditions, naturally occurring hybrids between *B. napus* and the related weedy species *Raphanus raphanistrum* and *Hirschfeldia incana* have been reported at very low frequencies (Darmency et al., 1998; Darmency and Fleury, 2000). According to the Australian Department of the Environment, *R. raphanistrum* (wild radish) is a serious agricultural weed widespread in all states and territories except the Northern Territory. *H. incana* (Buchan weed) is a common roadside weed found in Queensland, NSW, Victoria, Tasmania and South Australia (National weeds lists; accessed June 2017).

6.5 Presence of the introduced or similar genes and encoded proteins in the receiving environment

92. As discussed in Section 5.2.3, the seven introduced genes and their encoded proteins for production of LC-ω3-PUFAs were sourced from marine microalgae or yeast that are widespread and prevalent in the environment.

93. The microalgae *P. salina*, *M. pusilla* and *P. cordata* are commonly found in the ocean. People are naturally exposed to similar genes, their encoded proteins and the LC-PUFAs through contact with sea water and consumption of seafood such as fish and shellfish.

94. The yeasts *P. pastoris* and *L. kluyveri* are widely distributed in soil or on plants and fruits. People therefore naturally encounter the yeast genes and their encoded proteins through contact with soil and plants or consumption of fruits.

95. *The pat* gene was obtained from the common soil bacterium *S. viridochromogenes*. This is a saprophytic, soil-borne microbe that is not considered a pathogen of plants, humans or other animals (OECD, 1999). Genes encoding PAT or similar enzymes are present in a wide variety of bacteria. Acetyltransferases, the class of enzymes to which PAT belongs, are common enzymes in all microorganisms, plants and animals.

Section 7 Previous releases

7.1 Australian approvals of the GM canola line

96. DHA canola was approved by the Regulator for limited and controlled release under licence DIR 123 and has been grown in field trials in various local government areas in Victoria since 2014. The Regulator has not received any report of adverse effects as a result of this release.

7.2 Approvals by other Australian agencies

97. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. However, dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products.

98. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has determined that food derived from DHA canola is as safe for human consumption as food derived from conventional (non-GM) canola varieties (FSANZ, 2017b) and approved the use of food

derived from DHA canola in Australia, except that oil derived from DHA canola must not be used as an ingredient in infant formula products. This approval will take effect once the variation to Standard 1.5.2 of the Australia New Zealand Food Standards Code is completed.

99. The APVMA has regulatory responsibility for agricultural chemicals, including herbicides, in Australia. Nuseed has stated that, although DHA canola contains an herbicide tolerance gene conferring tolerance to glufosinate herbicides, the gene was used only as a selectable marker during the initial selection process in the laboratory. If there was any intention to use this trait in the cultivation of DHA canola, approval for glufosinate herbicide application must be obtained from the APVMA.

7.3 International approvals

100. Nuseed has obtained permits to conduct research trials of DHA canola in Canada and the United States of America. Nuseed carried out trials in Canada at two sites in Alberta and Saskatchewan in 2016 and three sites in Manitoba and Saskatchewan in 2017. Nuseed also carried out trials in the USA at one site in California in 2016 and at various sites in Oregon, Washington, Minnesota, North Dakota and South Dakota in 2017.

101. However, to date DHA canola has not been approved for commercial production and food/feed use in any country.

Chapter 2 Risk assessment

Section 1 Introduction

102. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 7). Risks are identified within the context established for the risk assessment (see Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

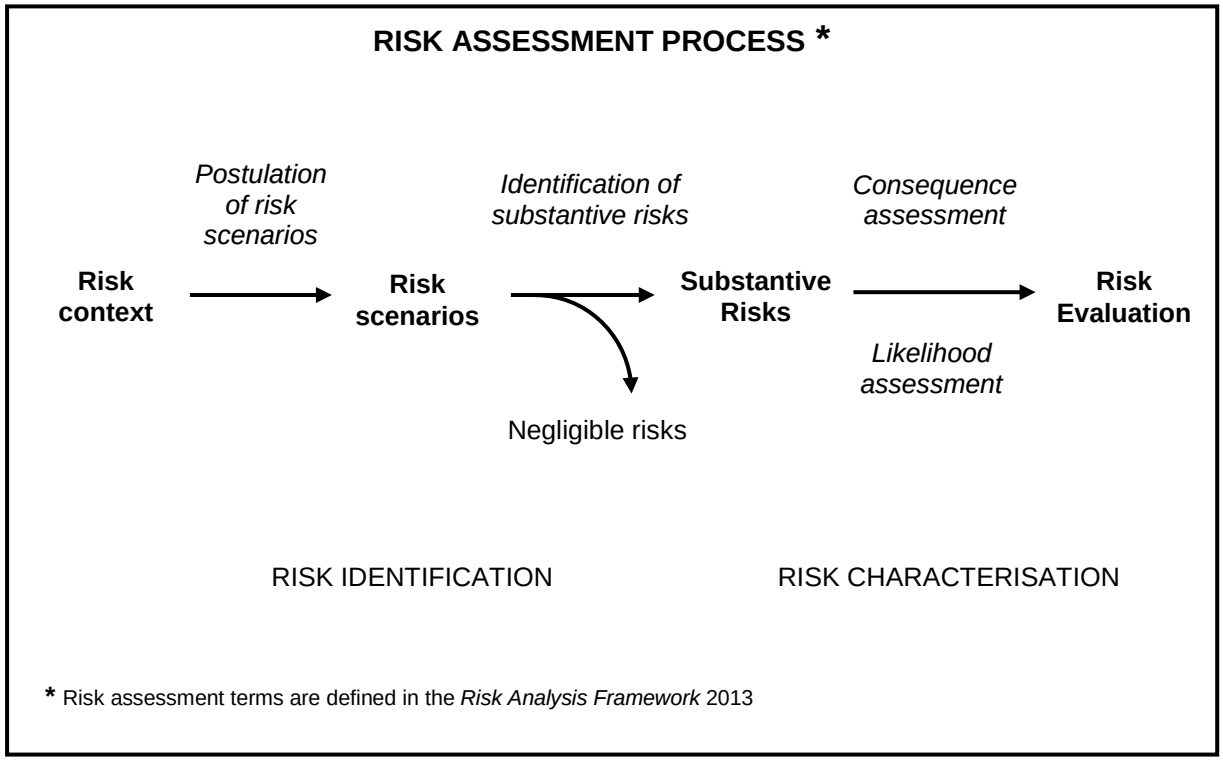


Figure 4 The risk assessment process

103. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO (risk scenarios) in the short and long term.

104. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, reported international experience and consultation (OGTR, 2013). A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants. In particular, novel traits that may increase the potential of the GMO to spread and persist in the environment or increase the level of potential harm compared with the parental plant(s) are considered in postulating risk scenarios (Keese et al., 2014). In addition, risk scenarios postulated in previous RARMPs prepared for licence applications for the same or similar GMOs are also considered.

105. Postulated risk scenarios are screened to identify those that are considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

106. Substantive risks (ie those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments. The level of risk, together with analysis of interactions between potential risks, is used to evaluate these risks to determine if risk treatment measures are required.

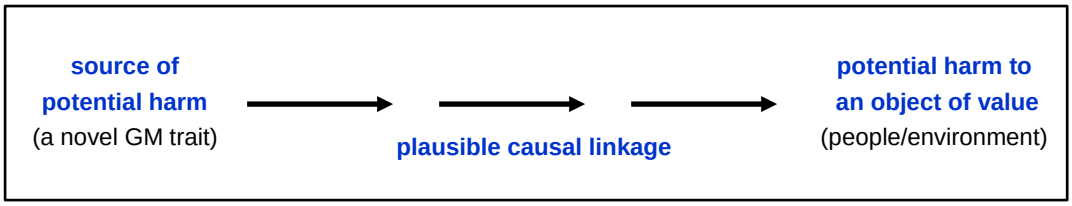
Section 2 Risk identification

107. Postulated risk scenarios are comprised of three components:

i. The source of potential harm (risk source)

ii. A plausible causal linkage to potential harm (causal pathway)

iii. Potential harm to an object of value (people or the environment).



108. When postulating relevant risk scenarios, the risk context is taken into account, including the following factors:

- the proposed dealings, which may be to conduct experiments, develop, produce, breed, propagate, grow, import, transport or dispose of the GMOs, use the GMOs in the course of manufacture of a thing that is not the GMO, and the possession, supply and use of the GMOs in the course of any of these dealings
- any proposed limits including the extent and scale of the proposed dealings
- any proposed controls to restrict the spread and persistence of the GMOs and
- the characteristics of the parent organism(s).

2.1 Risk source

109. The sources of potential harms can be intended novel GM traits associated with one or more introduced genetic elements, or unintended effects/traits arising from the use of gene technology.

The introduced genes for modified oil

110. As discussed in Chapter 1, DHA canola has been modified by the introduction of seven genes involved in a novel LC-ω3-PUFA pathway for DHA production in the seed. These introduced genes, their encoded proteins and the LC-ω3-PUFA pathway end product DHA are considered further as a potential source of risk.

The introduced gene for herbicide tolerance

111. DHA canola contains one selectable marker gene for glufosinate herbicide tolerance. This gene has been used in other GM canola approved for commercial release by the Regulator (Sections 5.2.2 and 6.4). GM canola lines expressing the PAT protein have been assessed to pose negligible risks to human health and the environment in the RARMPs for DIR 021/2002, DIR 108 and DIR 138 (OGTR, 2003, 2011, 2016a). The potential stacking of the *pat* gene with other herbicide tolerance genes in commercially approved GM canola (Roundup Ready®, TruFlex™ Roundup Ready® and Optimum™ GLY canola; tolerant to glyphosate herbicides) and non-GM canola (TT canola, tolerant to triazine herbicides; Clearfield® canola, tolerant to imidazolinone herbicides) to form multiple-herbicide tolerant hybrids has been assessed in the RARMPs for DIR 138 and DIR 139 as posing negligible risk

(OGTR, 2016a, b). Therefore, the potential risks associated with the *pat* gene in DHA canola will not be assessed further for this application.

The regulatory sequences

112. As discussed in Chapter 1, Section 5.3, the introduced genes are controlled by introduced regulatory sequences. These regulatory sequences are derived from common plants, a bacterium and plant viruses (see Table 2). Regulatory sequences are naturally present in plants, and the introduced elements are expected to operate in similar ways to endogenous elements. The regulatory sequences are DNA that is not expressed as a protein, and dietary DNA has no toxicity (Society of Toxicology, 2003). Hence, potential harms from the regulatory elements themselves will not be considered further for this application. However, the introduced regulatory sequences control gene expression and hence the distribution and concentration of the introduced proteins in the GM plants. The effects of protein levels, especially in relation to toxicity and allergenicity, will be considered below.

Unintended effects

113. The genetic modification has the potential to cause unintended effects in several ways including altered expression of endogenous genes by random insertion of introduced DNA in the genome, increased metabolic burden due to expression of the introduced protein, novel traits arising out of interactions with non-target proteins and secondary effects arising from altered substrate or product levels in biochemical pathways. However, these types of effects also occur spontaneously and in plants generated by conventional breeding. Accepted conventional breeding techniques such as hybridisation, mutagenesis and somaclonal variation can have a much larger impact on the plant genome than genetic engineering (Schnell et al., 2015). Plants generated by conventional breeding have a long history of safe use, and there are no documented cases where conventional breeding has resulted in the production of a novel toxin or allergen in a crop (Steiner et al., 2013). Therefore, unintended effects resulting from the process of genetic modification will not be considered further.

2.2 Causal pathway

114. The following factors are taken into account when postulating plausible causal pathways to potential harm:

- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the environment at the site(s) of release
- agronomic management practices for the GMOs
- spread and persistence of the GM plants (eg reproductive characteristics, dispersal pathways and establishment potential)
- tolerance to abiotic conditions (eg climate, soil and rainfall patterns)
- tolerance to biotic stressors (eg pests, pathogens and weeds)
- tolerance to cultivation management practices
- gene transfer to sexually compatible organisms
- gene transfer by horizontal gene transfer
- unauthorised activities.

115. Although all of these factors are taken into account, some are not included in risk scenarios because they are regulated by other agencies or have been considered in previous RARMPs (see sections 2.2.1 to 2.2.3, below).

2.2.1 Agronomic management and development of herbicide resistance

116. There is some potential for development of herbicide resistant weeds if a herbicide tolerant canola and its corresponding herbicide are used inappropriately. The repetitious use of a single herbicide, or herbicide group[4], increases the likelihood of selecting weeds that have developed herbicide resistance through natural mechanisms (Gressel, 2002). This is not a novel issue associated with this GMO, as most canola currently grown in Australia is herbicide tolerant, by either non-GM or GM mechanisms (see Chapter 1, Section 6.4).

117. The genetic modification to DHA canola also confers tolerance to glufosinate herbicides. The applicant has stated that the introduced *pat* gene was only used as a selectable marker during the initial selection process in the laboratory and is not intended to be used as a trait for breeding or any other purposes. As discussed in Section 2.1, GM canola lines containing the *pat* gene have been previously assessed to pose negligible risks to human health and the environment. Therefore, the issue of development of herbicide resistant weeds through selective pressure will not be further considered in this risk assessment.

2.2.2 Gene transfer by horizontal gene transfer

118. The potential for horizontal gene transfer from GMOs to other species that are not sexually compatible, and any possible adverse outcomes, has been reviewed in the scientific literature (Keese, 2008) as well as assessed in many previous RARMPs. Horizontal gene transfer was most recently considered in detail in the RARMP for DIR 108. This and other RARMPs are available from the GMO Record on the OGTR website or by contacting the OGTR. In previous assessments of horizontal gene transfer no substantive risk was identified, due to the rarity of these events and because similar gene sequences are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, horizontal gene transfer will not be assessed further.

2.2.3 Unauthorised activities

119. The potential for unauthorised activities to lead to an adverse outcome has been considered in previous RARMPs. In previous assessments of unauthorised activities, no substantive risk was identified. The Act provides for substantial penalties for unauthorised dealings with GMOs or non-compliance with licence conditions, and also requires the Regulator to have regard to the suitability of an applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, unauthorised activities will not be considered further.

2.3 Potential harm

120. Potential harms from GM plants include:

- harm to the health of people or desirable organisms, including toxicity/allergenicity
- reduced biodiversity for nature conservation
- reduced establishment or yield of desirable plants
- reduced products or services from the land use
- restricted movement of people, animals, vehicles, machinery and/or water
- reduced quality of the biotic environment (eg providing food or shelter for pests or pathogens) or abiotic environment (eg negative effects on fire regimes, nutrient levels, soil salinity, soil stability or soil water table).

[4] Herbicides are classified into groups based on their mode of action. All herbicide product labels must display the mode of action group. This enables users to rotate among herbicides with different modes of action to delay the development of herbicide tolerance in weeds.

121. These harms are based on those used to assess risk from weeds (Standards Australia et al., 2006; Keese et al., 2014). Judgements of what is considered harm depend on the management objectives of the land where the GM plant may be present. For example, a plant species may have different weed risk potential in different land uses such as dryland cropping or nature conservation.

2.4 Postulated risk scenarios

122. Four risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 4 and discussed individually below. Postulation of risk scenarios considers impacts of the GM canola or its products on people undertaking the dealings, as well as impacts on people and the environment exposed to the GM canola or its products as the result of the commercial use or the spread and persistence of plant material.

123. In the context of the activities proposed by the applicant and considering both the short and long term, none of the four risk scenarios gave rise to any substantive risks that could be greater than negligible.

Table 9 Summary of risk scenarios from the proposed dealings

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced LC-ω3-PUFA pathway genes	Commercial cultivation of GM canola expressing the LC-ω3-PUFA pathway genes ⬇ Exposure of humans and other desirable organisms by ingestion of GM canola oil, or contact with GM canola seed or products	Increased toxicity or allergenicity for humans OR increased toxicity to other desirable organisms	No	• The LC-ω3-PUFA pathway genes were sourced from yeasts and microalgae not known to be toxic to humans and other organisms • There is no known toxicity or allergenicity of the LC-ω3-PUFA pathway proteins and the end product DHA to humans or toxicity to animals and other organisms • The GM canola seed is compositionally equivalent to non-GM canola seed except for changed oil profile • Similar LC-ω3-PUFA pathway proteins and DHA are widespread in the environment • FSANZ has approved products derived from the GM canola for use in human food.
2	Introduced LC-ω3-PUFA pathway genes	Commercial cultivation of GM canola expressing the LC-ω3-PUFA pathway genes ⬇ Persistence of volunteer GM canola plants in agricultural areas OR dispersal of GM canola seed to intensive use	Reduced establishment or yield of desirable agricultural crops OR Reduced services from the land use	No	• Canola is not a persistent weed in agricultural areas or intensive use areas or nature reserves • The introduced genes are not expected to increase the potential weediness of the GM canola • Weed management strategies, including the

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
		areas or nature reserves ⬇ Establishment of volunteer/feral GM canola plants	OR Reduced biodiversity OR Increased reservoir for pests and pathogens		use of herbicides, can control volunteer GM canola • Most land managers of intensive use areas where volunteer/feral canola is present do not consider that canola warrants management.
3	Introduced LC-ω3-PUFA pathway genes	Commercial cultivation of GM canola producing seed containing DHA ⬇ GM canola seed consumed by pest animals ⬇ Increased fitness of pest animals ⬇ Impact of these animals on native or desirable vegetation	Reduced establishment of desirable vegetation OR Reduced biodiversity	No	• Exposure of pest animals and insects to DHA in the GM canola seed is low • Pests are controlled by current pest management practices
4	Introduced LC-ω3-PUFA pathway genes	Commercial cultivation of GM canola in agricultural areas ⬇ Cross-pollination with other canola crops, or sexually compatible Brassica crops or agricultural weeds ⬇ Establishment of hybrid GM canola plants or hybrid GM Brassica plants expressing the LC-ω3-PUFA pathway genes as volunteers ⬇ GM hybrids spread and persist	Increased toxicity or allergenicity in people or increased toxicity to other desirable organisms OR reduced establishment or yield of desirable plants	No	• Hybrids between the GM canola and other canola would be generated at low levels • Hybridisation between GM canola and Brassica crop species would occur at very low levels • Risk scenarios 1 and 2 did not identify toxicity, allergenicity or weediness of the GMO as substantive risks. Hybrids with sexually compatible plants are unlikely to differ • GM hybrids could be controlled by current weed management practices.

2.4.1 Risk scenario 1

Risk source	Introduced LC-ω3-PUFA pathway genes
Causal pathway	Commercial cultivation of GM canola expressing LC-ω3-PUFA pathway genes ⬇ Exposure of humans and other desirable organisms by ingestion of GM canola oil, or contact with GM canola seed or products ⬇
Potential harm	Increased toxicity or allergenicity for humans and increased toxicity to other desirable organisms

Risk source

124. The source of potential harm for this postulated risk scenario is the introduced LC-ω3-PUFA pathway genes.

Causal pathway

125. The applicant proposes that DHA canola would be cultivated on a commercial scale in the canola growing areas of Australia. The LC-ω3-PUFA pathway proteins have been confirmed to be present only in the DHA canola seed (see Chapter 1, Section 5.5.2).

126. The general public could be exposed to oil from the GM canola, if it were sold for human consumption. Processed canola oil does not contain detectable levels of protein but DHA canola oil contains high level of DHA, in addition to the other fatty acids normally present in non-GM canola oil.

127. People could be exposed to wind-borne GM canola pollen by inhalation. However, since expression of the introduced LC-ω3-PUFA pathway proteins is confined to the developing and mature seeds, this route of exposure to the introduced proteins does not apply to DHA canola.

128. People involved in cultivating or processing the GM canola, or using GM canola meal as animal feed, could be exposed to seeds or products through contact.

129. It is expected that GM canola seed meal would be routinely used as feed for livestock. Whole seeds could also occasionally be used as animal feed (OGTR, 2017a). In addition, GM canola could be grazed by livestock over winter if grown as a dual-purpose forage and grain crop (GRDC, 2009). Thus, livestock would be exposed to the LC-ω3-PUFA pathway proteins and DHA in the developing and mature seeds through feeding.

130. A number of other desirable organisms may also be exposed to LC-ω3-PUFA pathway proteins and DHA. Wild animals and birds could enter canola fields and feed on GM canola seed and soil organisms such as earthworms would contact decomposing seed after harvest. In addition, pollinators such as honeybees would be exposed to nectar and pollen from the GM canola. However, as expression of the introduced LC-ω3-PUFA pathway proteins is confined to the seed (see Chapter 1, Section 5.5.2), pollinators would have minimal or no exposure to these proteins, or to products of the pathway, through nectar and pollen.

Potential harm

131. Toxicity is the adverse effect(s) of exposure to a dose of a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Felsot, 2000). Allergenicity is the potential of a substance to elicit an immunological reaction following its ingestion, dermal contact or inhalation, which may lead to tissue inflammation and organ dysfunction (Arts et al., 2006).

132. The LC-ω3-PUFA pathway genes present in DHA canola are sourced from yeast and marine microalgae species that are not known to be toxic or pathogenic to humans, and the proteins encoded by these genes are not known to be toxic or allergenic and do not share relevant sequence homology with known toxins or allergens (Chapter 1, Section 5.2.3).

133. DHA canola seed contains varying levels of the LC-ω3-PUFAs, which are not naturally produced in non-GM canola. The end product of the LC-ω3-PUFA pathway, DHA, has been extensively studied and shown to have various beneficial effects on human health, with no report of toxicity or allergenicity (Chapter 1, Section 5.2.4). DHA is part of the normal human diet, as it is a common constituent of fish oils. A four-year clinical trial showed no identifiable risks associated with long-term consumption of DHA (Wheaton et al., 2003). FSANZ has approved the use of DHA rich oils from various marine microalgae as food or infant formula products in Australia.

134. As discussed in Chapter 1, Section 5.2.4, toxicity studies on rats showed that DHA had no observed adverse effects on the health of the test rats; while other studies on tree swallow and insect displayed beneficial effect of DHA on these organisms.

135. In Australia, non-GM canola varieties typically contain very low levels of two naturally occurring toxicants, erucic acid (less than 0.5%) and glucosinolates (less than 20 µmoles/g) (Colton and Potter, 1999), which are within the standard for canola oil (Oilseeds WA, 2006; CODEX, 2009). As discussed in Chapter 1, Section 5.5.3, compositional analysis of DHA canola seed showed no increased levels with biological significance for anti-nutrients, including glucosinolates and erucic acid, when compared to other non-GM canola varieties grown in Australia. Apart from the changed oil profile, DHA canola seed is compositionally equivalent to the seed of non-GM canola.

136. FSANZ has approved the use of food derived from DHA canola for human consumption in Australia (Chapter 1, Section 7.1).

137. Therefore, based on the known information, it is not expected that DHA canola would have increased toxicity or allergenicity to humans or increased toxicity towards other organisms.

Conclusion

138. Risk scenario 1 is not identified as a substantive risk because of the lack of toxicity or allergenicity of the introduced LC-ω3-PUFA pathway proteins or the end product DHA to humans, or toxicity to other desirable organisms. The GM canola seed is compositionally equivalent to non-GM canola seed except for the seed oil profile, proteins similar to LC-ω3-PUFA pathway proteins are widespread in the environment and FSANZ has approved food derived from the GM canola as safe for human consumption. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk Scenario 2

Risk source	Introduced LC-ω3-PUFA pathway genes
Causal pathway	Commercial cultivation of GM canola expressing the LC-ω3-PUFA pathway genes ⬇ Persistence of volunteer GM canola plants in agricultural areas OR dispersal of GM canola seed to intensive use areas or nature reserves ⬇ Establishment of volunteer/feral GM canola plants ⬇
Potential harm	Reduced establishment or yield of desirable agricultural crops OR Reduced services from the land use OR Reduced biodiversity OR Increased reservoir for pests and pathogens

Risk source

139. The source of potential harm for this postulated risk scenario is the introduced LC-ω3-PUFA pathway genes.

Causal pathway

140. The applicant proposes that DHA canola would be cultivated on a commercial scale. In current Australian agriculture, canola volunteers requiring weed management are likely to be found in fields for up to three years after growing a canola crop due to persisting seed banks (AOF, 2014). In contemporary German agricultural systems, canola volunteers were found up to fifteen years after harvest (Belter, 2016). Persisting canola seed banks have been shown to significantly contribute to the dynamics of feral canola populations (Pivard et al., 2008). As DHA canola is similar to non-GM

canola with respect to the intrinsic characteristics contributing to spread and persistence, such as seed production, shattering, dormancy, and competitiveness (Chapter 1, Section 5.5.3), it would be expected to produce similar numbers of volunteers.

141. After harvest, volunteer canola plants are also likely to occur following dispersal of GM canola seeds within agricultural areas due to pod shattering, strong winds, machinery movement and seed spillage during transport (OGTR, 2017a). As discussed in Chapter 1, Section 4.3, establishment of feral canola populations along transport routes or near processing or storage sites could occur due to seed spillage during transport. Occasionally whole seeds could be used as livestock feed and feral GM canola could potentially establish in animal feeding areas. If transport routes for harvested GM canola seeds passed through nature reserves, dispersal of canola seeds into the nature reserves could occur due to spillages. If GM canola fields were adjacent to nature reserves, short-range dispersal of canola seed into the nature reserves could occur due to movement of canola plant material from windrows by strong winds (Busi and Powles, 2016). Dispersal of viable canola seed into intensive use areas or nature reserves by endozoochory (consumption and excretion of seed) by wild mammals or birds is also possible at very low levels (Twigg et al., 2008).

142. Feral canola populations are thought to rely on re-supply of seed from spillages rather than forming self-sustaining weed populations. Surveys of roadside canola typically only found feral canola plants within 5 m of the edge of the road (Agrisearch, 2001; Norton, 2003). As discussed in Chapter 1, Section 4.3, canola is not considered a significant weed in natural undisturbed habitats in Australia (Dignam, 2001; Groves et al., 2003). As discussed (Chapter 1, Section 5.5.3), DHA canola is phenotypically similar to non-GM canola, apart from changes in seed oil profile, ie reduced oleic acid and a significant increase in PUFA content. Lower oleic acid content and higher PUFA content in canola seed oil has been shown to be associated with drought stress (Aslam et al., 2009). This may suggest that higher PUFA content in the seed could lead to better drought tolerance. Even if this is the case, it is not expected to alter the overall tolerance of DHA canola plants to biotic or abiotic stresses that normally restrict the geographic range and persistence of canola (Chapter 1, Sections 6.2 and 6.3). Therefore, feral DHA canola would not be expected to be more persistent or more invasive of natural habitats than non-GM canola.

143. DHA canola volunteers in agricultural areas could be controlled by current weed management practices. DHA canola is not different to its parental variety AV Jade or other non-GM canola varieties in that it can be readily controlled by glyphosate and 2,4-D amine herbicides (Leonforte, 2016) or other herbicide choices (AOF, 2014), except glufosinate.

Potential harm

144. Canola is a domesticated agricultural plant that has been the subject of management in Australia for decades. Volunteer canola is a weed of agricultural production systems (Simard et al., 2002; Groves et al., 2003). If left uncontrolled, volunteer canola plants could reduce the establishment or yield of desired crops. However, GM canola volunteers that are effectively controlled would not be expected to cause greater harm to desired crops than non-GM canola volunteers that are effectively controlled.

145. Volunteer canola could act as a reservoir for canola pests, pathogens or diseases. For example, blackleg is the most serious disease of canola in Australia, and over 95% of blackleg spores originate from the previous year's canola stubble (GRDC, 2009). Canola volunteers emerging in fields or field margins the year after a canola crop could be infected with blackleg from stubble, then in turn infect a canola crop planted in the following year. However, there is no difference in disease incidence between DHA canola and non-GM canola (Chapter 1, Section 5.5.3). GM canola volunteers that are effectively controlled would not be expected to cause greater harm as a disease reservoir than non-GM canola volunteers that are effectively controlled.

146. Feral canola on roadsides or along railway lines could potentially reduce services from the land use by obstructing lines of sight around corners and signs, as canola can grow to a height of 1.5 m (OGTR, 2017a). Also, the Western Australian Department of Parks and Wildlife lists feral canola as one of 60 weeds that threaten rail and roadside vegetation by lowering the biodiversity and aesthetic value of the verge, which are encouraged to be managed (Roadside Conservation Committee, 2014). However, feral canola is not listed under Weeds of National Significance.

147. A survey of 61 local councils and 25 road and rail authorities in canola growing regions of Australia found that approximately 30% of land managers identified feral canola as a weed present in their area, but approximately 70% of these land managers did nothing to control canola (Dignam, 2001), indicating that feral canola was not an issue of high priority.

148. If feral DHA canola populations were able to establish and persist in nature reserves, this could reduce the establishment of desirable native vegetation. It could give rise to lower abundance of desirable species, reduced species richness, or undesirable changes in species composition. However, the potential harms are no greater in DHA canola compared to its parental variety AV Jade and other non-GM canola, as the response of DHA canola to the abiotic and biotic factors (Chapter 1, Sections 6.2 and 6.3) that limit the ability of canola to spread and persist in natural habitats would be very similar to that of non-GM canola.

Conclusion

149. Risk Scenario 2 is not identified as a substantive risk because: 1) Standard weed management practices would control the GM canola volunteers in agricultural areas; 2) Canola is not a persistent weed in intensive use areas, weed management strategies can control feral GM canola, and most land managers of intensive use areas where feral canola is present do not consider it necessary to control canola; 3) Canola is not considered a significant weed in nature reserves, and the introduced genes do not increase the potential weediness of the GM canola. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk Scenario 3

Risk source	Introduced LC-ω3-PUFA pathway genes
Causal pathway	Commercial cultivation of GM canola producing seed containing DHA ⬇ GM canola seed consumed by pest animals ⬇ Increased fitness of pest animals ⬇ Impact of these animals on native or desirable vegetation ⬇
Potential harm	Reduced establishment of desirable vegetation OR Reduced biodiversity

Risk source

150. The source of potential harm for this postulated risk scenario is the introduced LC-ω3-PUFA pathway genes.

Causal pathway

151. The applicant proposes that DHA canola would be cultivated on a commercial scale. Thus, terrestrial pest animals such as rabbits, rats, mice or bird species or pest insects would be able to access DHA canola in the fields.

152. Terrestrial animals and insects do not normally have access to DHA in their food sources, as DHA is usually found in marine algae, aquatic insects or fish. Thus, commercial plantation of DHA

canola will make it possible for these animals or insects to ingest DHA if they feed on the GM canola seed. Larger pest animals such as rabbits would have lower exposure, as they generally only eat vegetative tissues such as leaves that do not contain DHA, but pest birds such as common mynas (*Acridotheres tristis*) eat seeds and fruits when they do not have sufficient invertebrate food. Mice usually feed on the nutrient-rich plant parts including growing points, flowers and seeds. On broadacre properties, where crops would be the main food source for rodent pests, it is possible that the GM canola could be the predominant food source of rodents. As discussed in Chapter 1, Section 5.2.4, research to date has not revealed any toxicity of DHA to animals or insects. Rather, for many species it may have a beneficial role in their growth and general health. Thus it could be posited that availability of additional exogenous DHA in the diet may potentially lead to an increase in fitness, with a resulting impact on surrounding vegetation.

Potential harm

153. As detailed in Chapter 1, Section 5.2.4, American tree swallow chicks fed with diets containing higher amount of EPA and DHA, where food was limiting, were shown to be healthier compared to chicks with lower amount of EPA and DHA. However, this species has evolved to live on a mixed aquatic and terrestrial diet which contains DHA and therefore may not be able to synthesis it from precursors. It is thought likely that terrestrial mammals that have evolved far from sources of DHA, can synthesise LC-PUFAs from precursors (Martinez Del Rio and McWilliams, 2016).

154. While there is a body of literature that supports the importance of omega-3 fatty acids in maintaining growth and reproductive health in terrestrial animals, the positive effect of supplementation is unclear for animals capable of synthesising LC-PUFAs. Thus, a study on the effect of dietary DHA on biosynthesis of DHA from α-linolenic acid in young rats indicated that dietary DHA intake may be important for maintaining the functions of the heart, lungs, kidneys and spleen for rats (DeMar et al., 2008). However, there is no data to suggest enhanced fitness or competiveness.

155. If consumption of seed from DHA canola were to enhance the environmental fitness of pest animals, this could lead to a greater impact of these animals on native or desirable vegetation or increased competition for desirable animals/birds. However, other food sources such as aquatic insects (Twining et al., 2016), some seeds, leaves and nuts already contain omega 3 fatty acids and in agricultural cropping areas food is not limiting for pest species. In the case of broadacre crops, rodents are subjected to control measures including maintaining crop hygiene to reduce rodent numbers, monitoring rodent activity and baiting. These are used routinely to minimise crop loss from pests (GRDC, 2011). This would effectively reduce the chance for rodent populations to access DHA in DHA canola crops. Thus the availability of additional omega-3 fatty acids through consumption of DHA canola is unlikely to change the existing impact of known pest animals.

156. If pest insects fed on DHA canola could become stronger and more competitive (Chapter 1, Section 5.2.4), they may cause more damage to other crops in agriculture areas, or reduce native or desirable vegetation and increased competition for desirable insects. However, pest insect infestations on canola mainly feed on vegetative tissues and no pest insects feed solely on canola seed (GRDC, 2009). Therefore, the chance for pest insects to access DHA in the DHA canola seed is very low. In addition, pest insects in canola fields are readily controlled by current pest management practices, including the application of various insecticides (Hertel and Roberts, 2007).

Conclusion

157. Risk scenario 3 is not identified as a substantive risk because exposure of pest animals and insects to DHA in the GM canola seed is low and pests are controlled by pest management practices in the canola fields. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.4 Risk Scenario 4

Risk source	Introduced LC-ω3-PUFA pathway genes
Causal pathway	Commercial cultivation of GM canola expressing the LC-ω3-PUFA pathway genes ⬇ Cross-pollination with other canola crops, or sexually compatible Brassica crops or agricultural weeds ⬇ Establishment of hybrid GM canola or hybrid GM Brassica plants expressing the LC-ω3-PUFA pathway genes as volunteers ⬇ GM hybrids spread and persist ⬇
Potential harm	Increased toxicity or allergenicity in people or increased toxicity to other desirable organisms OR reduced establishment or yield of desirable plants

Risk source

158. The source of potential harm for this postulated risk scenario is the introduced LC-ω3-PUFA pathway genes.

Causal pathway

159. The applicant proposes that DHA canola would be cultivated on a commercial scale in the canola growing areas of Australia. The LC-ω3-PUFA pathway genes could potentially be transferred by pollen flow to other commercially grown canola. This could also bring it into proximity to other Brassica crop species, such as vegetables, forage crops and Indian mustard, as well as related weeds.

Interactions with other canola crops

160. Cross pollination between the GM canola proposed for release and other canola would most likely occur when different canola crops are grown in adjacent paddocks and flower synchronously. Cross pollination may also occur at a smaller scale with volunteer or feral canola populations.

161. Outcrossing rates between neighbouring commercial canola fields in Australia are less than 0.1% averaged over whole fields (Rieger et al., 2002). Correspondingly low levels of hybridisation are expected between the GMO and other non-GM and GM canola detailed in Chapter 1, Section 6.4. Since the introduced LC-ω3-PUFA pathway genes are specifically expressed in the seed of DHA canola (Chapter 1, Section 5.5.2), the pollination characteristics of DHA canola are therefore not expected to change compared to non-GM canola or to other GM canola previously approved for commercial cultivation.

162. Hybrid seed with the GM trait could disperse within agricultural areas, to intensive use areas, or to nature reserves, by the same mechanisms described in Risk Scenarios 2. In addition, if a field that is adjacent to the DHA canola field is planted with an open pollinating (OP) canola variety, the farmer may retain seed, including a proportion of GM hybrid seed, for future planting. In 2015, 98% of the TT canola area was sown to OP varieties, while 98% the Roundup Ready® area and 75% of the Clearfield® canola were sown to hybrid varieties (DAF-WA, 2017). Open pollinated TT canola varieties still remain the most important group for Australian canola growers.

Interactions with Brassica crop species

163. Pollen flow between the GM canola proposed for release and other Brassica crop species could occur if the Brassica crops were grown in proximity to the GM canola and flowered synchronously. Brassica vegetable crops are generally harvested prior to flowering unless they are grown for seed production, in which case precautions would usually be taken to avoid crossing with oilseed canola (OGTR, 2017a). Brassica forage crops rarely flower due to heavy grazing. *B. juncea* (Indian mustard)

crops, which are grown as oilseeds or for condiment mustard, could plausibly cross-pollinate with the GM canola. Cross pollination could also conceivably occur with volunteer populations of Brassica plants.

164. Hybrids between *B. napus* and *B. juncea* have been observed in the field, are fertile, and often have high fitness (Liu et al., 2010). Cross-pollination between *B. napus* and *B. rapa* occurs frequently in the field if plants of the two species are in proximity, and the hybrids are vigorous and fertile, although with reduced pollen viability (Warwick et al., 2003). Hybrids between *B. napus* and *B. oleracea* have been detected at low levels in wild populations (Ford et al., 2006).

165. Based on the data above, hybridisation between GM canola and other Brassica crop species is expected to occur if the GM canola is released. However, the frequency of interspecies crossing would be lower than the frequency of crossing between the GM canola and other canola plants, both because there is greater sexual compatibility between *B. napus* plants than between *B. napus* and other species, and because canola is far more widely grown than other Brassica crops (ABARES, 2017). Since hybridisation between GM canola and other canola would occur at low levels, hybridisation between GM canola and other Brassica crop species is likely to occur at very low levels.

166. Volunteer plants that are hybrids between GM canola and other Brassica crop species could be controlled by standard weed management practices include using herbicides approved by the APVMA for use on Brassica volunteers. As discussed in Risk scenario 2, the presence of the LC-ω3-PUFA pathway genes is not expected to alter intrinsic characteristics contributing to spread and persistence, or to alter the tolerance of GM plants to biotic or abiotic stresses. Therefore, GM hybrid volunteers would not be expected to be more invasive or persistent than hybrids between non-GM canola and other Brassica crop species.

Interactions with Brassicaceae weeds

167. Brassicaceae agricultural weeds are expected to be present in fields or field margins where GM canola would be grown. Cross-pollination could occur if weeds are not destroyed by weed management prior to flowering, if there is synchronous flowering of weeds and the crop, and if the weed species is sexually compatible with *B. napus*.

168. Cross-pollination between *B. napus* and wild radish (*R. raphanistrum*) has been observed in the field at very low levels. The hybrids are smaller than either parent and close to sterile (Darmency et al., 1998; Warwick et al., 2003). Cross-pollination between *B. napus* and Buchan weed (*H. incana*) has been observed in the field at low levels. The hybrids had very low fertility, and by the fifth generation of back-crossing the progeny produced no viable seed (Darmency and Fleury, 2000). Thus, introgression of the LC-ω3-PUFA pathway genes from GM canola into wild radish or Buchan weed populations is highly unlikely.

169. *B. napus* has been reported to cross with other Brassicaceae weeds with human intervention, but not in open-pollination field conditions. Therefore, hybridisation between the GM canola and other Brassicaceae weeds would be highly unlikely.

170. In the highly unlikely event that the LC-ω3-PUFA pathway genes was introgressed into populations of wild radish or Buchan weed, the GM weeds are not expected to be more weedy than the parent non-GM weeds, as the presence of the LC-ω3-PUFA pathway genes is not expected to alter intrinsic characteristics contributing to spread and persistence as discussed above.

Potential harm

Interactions with other canola crops

171. Transfer and expression of the introduced genes could alter the potential toxicity and/or allergenicity of the resulting plants, or reduced establishment or yield of desirable plants through increased weediness of GM hybrids.

172. As discussed in Risk scenario 1, allergenicity to people and toxicity to people and other organisms are not expected to be changed by the introduced LC-ω3-PUFA pathway genes, nor would any change be expected in GM hybrids.

173. As discussed in Risk scenario 2, the genes introduced into the hybrid GM canola plants are not expected to alter the tolerance of plants to biotic or abiotic stresses that normally restrict geographic range and persistence of canola in natural habitats. Similarly, they would not be expected to alter the geographic range or persistence of other canola plants if the introduced LC-ω3-PUFA pathway genes were transferred to hybrids or their progeny.

174. Transfer and expression of the introduced genes may also lead to hybrids with altered oil composition and potentially affect the specific oil characteristics of other canola. For example, in Australia, speciality canola varieties with high stability seed oils (ie high in oleic acid and low in linolenic acid) for high quality canola oil production have been grown since 2006 (Salisbury et al., 2016). However, this would not lead to increased level of toxicity or allergenicity of the resulting hybrids.

Interactions with Brassica crop species

175. Any hybrid between the GM canola and other Brassica crop species expressing the introduced genes could also potentially have increased toxicity and/or allergenicity to people, or increased weediness potential. As discussed in Risk scenario 1, the introduced genes do not encode proteins that are considered toxic or allergenic. Therefore, even if the introduced genes were to be transferred to, and expressed in, Brassica crop species, the recipient species would likely be no more toxic or allergenic than their unmodified precursors.

176. Both volunteer canola and other Brassica crop species are weeds of agricultural production systems (Groves et al., 2003). Any hybrids between the GM canola and other Brassica species could also potentially become volunteers. If left uncontrolled, GM hybrid volunteers could reduce the establishment or yield of desired crops. However, under the current weed management practices, GM hybrid volunteers would not cause more harm than hybrids between non-GM canola and other Brassica crop species.

Interactions with Brassicaceae weeds

177. According to the Australian Department of the Environment, wild radish and Buchan weeds are both declared weeds in canola growing states and are not easily controlled in agricultural areas (National weeds lists; accessed August 2017). If the LC-ω3-PUFA pathway genes were introgressed into populations of these weeds and increased their potential for spread and persistence, these GM weeds could have more impact on the agricultural environment by reducing the establishment or yield of desired crops. However, as discussed in Risk Scenario 2, DHA canola is not identified as weedier than non-GM canola. Therefore, under the current weed management practices, these GM hybrids would not cause more harm than hybrids between non-GM canola and wild radish and Buchan weeds.

Conclusion

178. Risk scenario 4 is not identified as a substantive risk because hybrids between DHA canola and other canola or sexually compatible plant species would be generated at low levels; the GM hybrids are not expected to have increased allergenicity to people and the hybrids can be controlled by standard weed management practices. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

179. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis[5]. There are several types of uncertainty in risk analysis (Bammer & Smithson 2008; Clark & Brinkley 2001; Hayes 2004). These include:

- uncertainty about facts:
  - knowledge – data gaps, errors, small sample size, use of surrogate data
  - variability – inherent fluctuations or differences over time, space or group, associated with diversity and heterogeneity
- uncertainty about ideas:
  - description – expression of ideas with symbols, language or models can be subject to vagueness, ambiguity, context dependence, indeterminacy or under-specificity
  - perception – processing and interpreting risk is shaped by our mental processes and social/cultural circumstances, which vary between individuals and over time.

180. Uncertainty is addressed by approaches including balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios involving uncertainty to lead to harm. If there is residual uncertainty that is important to estimating the level of risk, the Regulator will take this uncertainty into account in making decisions.

181. DHA canola was approved by the Regulator for limited and controlled release (field trials) under licence DIR 123. The RARMP for DIR 123 identified additional information that could be required to assess a large scale or commercial release of the GM canola lines. This included additional molecular and biochemical characterisation of the GM canola lines, particularly with respect to production of potential toxins or allergens, and additional phenotypic characterisation of the GM canola line, particularly with respect to traits that may contribute to weediness. Information provided by the applicant to address these areas of uncertainty is presented and discussed in Chapter 1, Sections 5.5.1 (molecular characterisation), 5.2.3 (toxicity and allergenicity of the yeast and microalgal proteins) and 5.2.4 (toxicity and allergenicity of DHA) and Chapter 1, Section 5.5.3 (phenotypic and agronomic characterisation).

182. Uncertainty can arise from a lack of experience with the GMO. DHA canola has not yet been grown commercially anywhere in the world. However, the level of uncertainty is considered to be low given that extensive field trials have been conducted in the United States, Canada and Australia. The uncertainty has been taken into account in assessment of risk scenarios, and is not sufficient to affect the conclusions on the overall level of risk.

183. For commercial releases of GMOs, which typically do not have limited duration, uncertainty regarding any future changes to knowledge about the GMO is addressed through post release review (Chapter 3, Section 4).

Section 4 Risk evaluation

184. Risk is evaluated against the objective of protecting the health and safety of people and the environment to determine the level of concern and, subsequently, the need for controls to mitigate or reduce risk. Risk evaluation may also aid consideration of whether the proposed dealings should be authorised, need further assessment, or require collection of additional information.

185. Factors used to determine which risks need treatment may include:

[5] A more detailed discussion of uncertainty is contained in the Regulator's *Risk Analysis Framework* available from the OGTR website or via Free call 1800 181 030.

- risk criteria
- level of risk
- uncertainty associated with risk characterisation
- interactions between substantive risks.

186. Four risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. The level of risk for each scenario was considered negligible in relation to both the seriousness and likelihood of harm, and by considering both the short and long term. The principal reasons for these conclusions are summarised in Table 9.

187. The *Risk Analysis Framework* (OGTR, 2013), which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.

Chapter 3 Risk management plan

Section 1 Background

188. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan addresses risks evaluated as requiring treatment and considers limits and controls proposed by the applicant, as well as general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through proposed licence conditions.

189. Under section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment.

190. All licences are subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings. Section 64 requires the licence holder to provide access to premises to OGTR inspectors and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder must also be reported to the Regulator.

191. The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings and to manage risk to people or the environment. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Risk treatment measures for substantive risks

192. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed release of DHA canola. These risk scenarios were considered in the context of the large scale of the proposed release and the receiving environment. The risk evaluation concluded that no controls are required to treat these negligible risks.

Section 3 General risk management

193. All DIR licences issued by the Regulator contain a number of conditions that relate to general risk management. These include conditions relating to:

- applicant suitability
- testing methodology
- identification of the persons or classes of persons covered by the licence
- reporting requirements and
- access for the purpose of monitoring for compliance.

3.1 Applicant suitability

194. In making a decision whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under section 58 of the Act, matters that the Regulator must take into account include:

- any relevant convictions of the applicant (both individuals and the body corporate)

- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- the capacity of the applicant to meet the conditions of the licence.

195. On the basis of information submitted by the applicant and records held by the OGTR, the Regulator considers Nuseed suitable to hold a licence. The licence includes a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability.

196. In addition, any applicant organisation must have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

3.2 Testing methodology

197. Nuseed is required to provide a method to the Regulator for the reliable detection of the GMO, and the presence of the introduced genetic materials in a recipient organism. This instrument is required prior to conducting any dealings with the GMO.

3.3 Identification of the persons or classes of persons covered by the licence

198. Any person, including the licence holder, may conduct any permitted dealing with the GMO.

3.4 Reporting requirements

199. The licence requires the licence holder to immediately report any of the following to the Regulator:

- any additional information regarding risks to the health and safety of people or the environment associated with the dealings
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the release.

200. The licence holder is also obliged to submit an Annual Report containing any information required by the licence.

201. There are also provisions that enable the Regulator to obtain information from the licence holder relating to the progress of the commercial release (see Section 4, below).

3.5 Monitoring for compliance

202. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow the Regulator, or a person authorised by the Regulator, to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

203. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. The Act provides for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to the health and safety of people or the environment could result.

Section 4 Post release review

204. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator takes account of the likelihood and impact of an adverse outcome over the foreseeable future, and does not disregard a risk on the basis that an adverse outcome might only occur in the longer term. However, as with any predictive process, accuracy is often greater in the shorter rather than longer term.

205. The Regulator has incorporated a requirement in the licence for ongoing oversight to provide feedback on the findings of the RARMP and ensure the outcomes remain valid for future findings or changes in circumstances. This ongoing oversight will be achieved through post release review (PRR) activities. The three components of PRR are:

- adverse effects reporting system (Section 4.1)
- requirement to monitor specific indicators of harm (Section 4.2)
- review of the RARMP (Section 4.3).

206. The outcomes of these PRR activities may result in no change to the licence or could result in the variation, cancellation or suspension of the licence.

4.1 Adverse effects reporting system

207. Any member of the public can report adverse experiences/effects resulting from an intentional release of a GMO to the OGTR through the Free-call number (1800 181 030), fax (02 6289 4404), mail (MDP 54 – GPO Box 9848, Canberra ACT 2601) or via email to the OGTR inbox (ogtr@health.gov.au). Reports can be made at any time on any DIR licence. Credible information would form the basis of further investigation and may be used to inform a review of a RARMP (see Section 4.3 below) as well as the risk assessment of future applications involving similar GMOs.

4.2 Requirement to monitor specific indicators of harm

208. Collection of additional specific information on an intentional release provides a mechanism for 'closing the loop' in the risk analysis process and for verifying findings of the RARMP, by monitoring the specific indicators of harm that have been identified in the risk assessment.

209. The term 'specific indicators of harm' does not mean that it is expected that harm would necessarily occur if a licence was issued. Instead, it refers to measurement endpoints which are expected to change should the authorised dealings result in harm. The licence holder is required to monitor these specific indicators of harm as mandated by the licence.

210. The triggers for this component of PRR may include risk estimates greater than negligible or significant uncertainty in the risk assessment.

211. The characterisation of the risk scenarios discussed in Chapter 2 did not identify any risks greater than negligible. Therefore, they were not considered substantive risks that warranted further detailed assessment. Uncertainty is considered to be low. No specific indicators of harm have been identified in this RARMP for application DIR 155. However, specific indicators of harm may also be identified during later stages, eg through either of the other components of PRR.

212. Conditions have been included in the licence to allow the Regulator to request further information from the licence holder about any matter to do with the progress of the release, including research to verify predictions of the risk assessment.

4.3 Review of the RARMP

213. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would take into account any relevant new information, including any changes in the context of the release, to determine if the findings of the RARMP remained current. The timing of the review would be determined on a case-by-case basis and may be triggered by findings from either of the other components of PRR or be undertaken after the authorised dealings have been conducted for some time. If the review findings justified either an increase or decrease in the initial risk estimate(s), or identified new risks to people or to the environment that require management, this could lead to changes to the risk management plan and licence conditions.

Section 5 Conclusions of the RARMP

214. The risk assessment concludes that the proposed commercial release of GM canola poses negligible risks to the health and safety of people or the environment as a result of gene technology, and that these negligible risks do not require specific risk treatment measures.

215. However, general conditions have been imposed to ensure that there is ongoing oversight of the release.

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Appendix A Summary of submissions from prescribed experts, agencies and authorities

The Regulator received a number of submissions from prescribed experts, agencies and authorities on matters considered relevant to the preparation of the RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. The issues raised, and how they are addressed in the consultation RARMP, are summarised below.

Submission	Summary of issues raised	Comment
1	Agrees with the issues identified by the office for consideration in the RARMP and no new issues were identified for consideration.	Noted
2	No comment, as Council does not have a specialist scientific expert to make an assessment.	Noted
3	No comment as the Shire is not subject to the growing of canola within the Shire boundary.	Noted
4	No comment as there is not any land within Council boundaries that could sustain commercially grown crops.	Noted
5	Advises that Council: a) prefers the Council area be GMO free b) does not support the commercial release of GM canola. Before the commercial release is allowed the following concerns need to be addressed: · commercial impact on overseas markets on our product · effective segregation available · a caveat requiring GM companies to compensate farmers and businesses for unintended consequences of the release. c) Does not support trials in Council area but if they are to occur then the company conducting the trial should: · Advise Council of the trial sites · Advise all neighbouring farmers with properties and apiarists with bees within 3 km of those sites. Ensure that harvesting and carriage of seed produced is controlled to prevent any escape of seed.	Noted. This is an application for commercial release and if a licence were issued the GM canola could potentially be grown in all canola growing areas in Australia (as for other non-GM canola or commercially approved GM canola). This may be subject to restrictions imposed by some States and Territories for marketing reasons. States and Territories may allow trials of GM crops subject to conditions unrelated to human health and safety and the environment. Marketing, commercial liability and trade issues, including segregation and coexistence regimes, are outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence. These are matters for States and Territories, and industry. The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology. The RARMP for this commercial release concludes that risks to human health and the environment are negligible. Therefore, only general conditions are included in the draft licence, to ensure that there is ongoing oversight of the release.
6	Council does not have in house expertise relating to genetic modification, so is not in a position to comment on the potential environmental and health impacts of the	Noted

Submission	Summary of issues raised	Comment
	proposed release.	
7	Comments that there appears to be limited information on the use of canola as a food source by native animals (and hence exposure of native animals to the altered chemical constituents of the material). Notes that the promoters of the seven introduced genes that confer the altered oil profile are seed specific so pollinators may have minimal exposure to the expressed proteins and the fatty acids. Suggests that tissue specificity of these promoters needs to be thoroughly addressed in the RARMP, together with the inclusion of any information on the use by animals of canola as a food source.	Use of canola as a food source by native animals is discussed in Chapter 2, Section 2.4 (Risk scenario 3). Seed specificity of the introduced proteins for LC-PUFA production is discussed in Chapter 1, Section 5.5.2 and Chapter 2, Section 2.4 (Risk Scenario 1).
	As this is a commercial release, recommends that the toxicity of each of the introduced proteins be analysed against criteria such as: · History of safe use · Bioinformatics data · Mode of action · Digestibility of the proteins If the 'weight-of-evidence' of an evaluation of such criteria suggests the protein is safe, then data from a 'higher tier' study (such as examining acute toxicology) may not be necessary. The toxicity of the selectable marker gene (*pat*) protein product should be also addressed in this RARMP. Given that the aim of the genetic modifications is to produce long chain omega-3 polyunsaturated fatty acids in the GM plants, the potential toxicity of these compounds and their biosynthetic precursors should be reviewed, together with the possibility that they may increase the fitness of animals (both desirable animals and pests). The risk assessment should also note whether the introduced genes come from any organism that has been associated with toxic, allergenic, or pathogenic properties in other organisms in the environment.	Potential for the introduced proteins for LC-PUFA production to have toxic properties or for the proteins to catalyse the production of a toxic metabolite in the GM plants is discussed in Chapter 1 Section 5.2.3, and Chapter 2, Section 2.4 (Risk Scenario 1). These sections also include comment on the source organisms. Chapter 2, Risk scenario 3 discusses the possibility that the change in GM canola oil profile resulting from the genetic modification could lead to increased fitness of ingesting animals. Potential toxicity resulting from the introduced *pat* gene is discussed in Chapter 1, Section 5.2.2 and Chapter 2, Section 2.1.
	B. napus is not recorded in the Australian government's 'Weeds of National Significance' list, the 'National Environment Alert List', or the 'Noxious Weed List for Australian States and Territories'. It can be a problem in agricultural systems, but is only a minor problem in natural ecosystems. It is recommended that the RARMP thoroughly cover both the factors that restrict the ability of canola (unmodified and currently	These issues are discussed in Chapter 1, Sections 6.2 and 6.3; Chapter 2, Section 2.4 (Risk scenarios 2).

Submission	Summary of issues raised	Comment
	commercially released GM lines) to spread and persist in natural ecosystems, and the potential for the genetic modification to increase the ability of the GM plants to spread and persist.	
	It is noted that *B. napus* is sexually compatible with a number of other cultivated Brassica species such as *B. juncea* and *B. rapa*. Further, at low frequencies it can hybridise with weedy members of the Brassicaceae family, most notably wild radish, Buchan weed and charlock. The likelihood for gene flow, and the potential adverse effects to the environment of the introgression of the omega-3 oil trait into any other species, should be covered in the RARMP.	The possibility of gene transfer and the potential adverse effects to the environment are addressed in Chapter 1 Section 6.3 and Chapter 2, Section 2.4 (Risk scenario 4).
	There is extensive experience in the general management of canola in agricultural settings, including GM canola that has been engineered for herbicide tolerance and the management of volunteers in natural ecosystems. This experience should be directly applicable to the management of the GM plants in this application, and therefore it is recommended that it is discussed in the RARMP.	General management of the GM canola in agricultural settings is discussed in Chapter 1, Section 6.1.
8	Overall Nuseed's application has negligible risks to the health and safety of people and the environment. Specifically, the proposed modification to canola is highly unlikely to increase the species' weed risk to native vegetation and grazing land.	Noted
	General questions to consider: · Is the non-GM parent strain grown commercially in Australia? · Is the seed production and dispersion of the seeds of the GM plants similar to that of the wild-type parent? · What strategies will be in place, such as buffer zones and control of volunteer plants, to ensure the GM canola does not become established in the environment? · Apart from glufosinate, what other commercially available herbicides does the GM canola remain susceptible? · Has any herbicide susceptibility been tested or is it assumed based on the parental strain?	The RARMP (Chapter 1) notes that the parent cultivar, AV Jade, is not commercially grown in Australia. Weediness of the GM canola is addressed in Chapter 1, Section 5.5.3 and Chapter 2 Section 2.4 (Risk scenario 2). This is a licence application for commercial release of the GM canola. Since the RARMP concludes that the GMO poses no greater risks to human health and the environment than non-GM canola, no specific draft licence conditions were proposed to contain the GM canola. Volunteer plants from the GM canola would be controlled by the standard weed management practices the same as non-GM canola. The GM canola has been tested to have the same susceptibility to glyphosate and 2,4-D as its parental variety and this is discussed in Chapter 1, Section 6.1.

Appendix B Summary of advice from prescribed experts, agencies and authorities on the consultation RARMP

The Regulator received a number of submissions from prescribed experts, agencies and authorities on the consultation RARMP. All issues raised in submissions that related to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence and were used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence. Advice received is summarised below.

Submission	Summary of issues raised	Consideration in RARMP	Comment
1	No comment	-	Noted
2	No comment	-	Noted
3	Council has no canola growing areas in its district and does not have an official policy concerning GM products. However, any proposed release should be undertaken in a way that is safe to both the public and the environment.	-	Noted
4	No comment	-	Noted
5	Council does not have the specialist scientific expertise available to comment in any detail on the risks of a GM crop trial in the region but notes that a council resolution was passed in 2001 declaring the municipality to be a GM-free district.	-	Noted
	Council's principal concern is the generation of weed species that are difficult to control. There are many significant environmental assets within the Shire and weeds pose a major thread to their integrity. Council would be concerned if GM canola was planted adjacent, or within easily vectored distance, to some of the areas of significant biodiversity. Many of these are captured in the roadsides and invasion of canola crops without practical control measures available would be disastrous.	Chapters 1 and 2	The RARMP concludes that risks to people and the environment from this commercial release are negligible. The risk of weediness for the GM canola to be released has been assessed to be no greater than the currently grown non-GM canola varieties.
	Council's commitment to a clean and green image is demonstrated by the significant number of organic farmers in the region. Council is very careful when carrying out weed control works and always ensures	-	Marketing issues, including declaring areas to be GM free for marketing purposes, are the responsibility of the States and Territories.

Submission	Summary of issues raised	Consideration in RARMP	Comment
	that any chemical spraying has no impact on the farmer's organic accreditation.		
	Council cannot give any useful feedback on local issues without more details on the location of the release site, particularly in relation to areas of biodiversity or organic farms.	-	This is an application for commercial release and if a licence were issued the GM canola could potentially be grown in all canola growing areas in Australia (as for other non-GM canola or commercially approved GM canola), except for some areas with restrictions imposed by States and Territories for marketing reasons.
6	Agrees with the overall conclusions of the consultation RARMP that the risks to the environment are negligible.	-	Noted
	Paragraph 142 in Risk scenario 2 refers to Chapter 1, Section 4.1 as a reference that canola has not been considered as a 'significant weed in natural undisturbed habitats in Australia'. A more appropriate reference would be Chapter 1, Section 4.3.	Chapter 2	Paragraph 142 has been amended accordingly.
	Suggests that further discussion of the weediness of canola in natural habitats, including any information on the frequency of populations of canola in natural habitats, be included in Risk scenario 2. Should emphasise that canola is considered to be a domesticated agricultural plant that has been the subject of management in Australia for decades. Paragraph 143 indicates that the management of the GM plants in agricultural areas would depend on current weed management practices, suggests also stating that the management of the GM plants in natural habitats would also depend on such practices.	Chapter 2	Based on the currently available information, canola has not been identified as a problematic weed in natural habitats both in Australia and overseas and no data on the frequency of populations in natural habitats can be found. However, some changes in wording have been made as suggested. Note that current weed management practices do not apply to the management of weeds in natural habitats in Australia.
	Reference to Australian government websites dealing with weeds in Australia would be useful in discussing the agricultural plant status/non-weed status of canola in Australia.	Chapter 2	Reference to the Weeds of National Significance website has been included in Risk scenario 2.
	Paragraph 148 states that the 'potential harms' of the GM canola	Chapter 2	Text has been amended accordingly.

Submission	Summary of issues raised	Consideration in RARMP	Comment
	in natural habitats 'are no greater in DHA canola compared to the parental variety'. This statement needs to be followed by supporting evidence (or referral to elsewhere in the RARMP for supporting evidence).		
7	The consultation within our government agencies indicates that overall this application has negligible risks to the health and safety of people and the environment. Specifically, unmodified canola has low weed risk and the proposed genetic modification is unlikely to change its weed risk to native vegetation and grazing land.	-	Noted
8	Notes that the licence is for the same product that is the subject of a current FSANZ application, being A1143 (food derived from DHA canola line BS-B50027-4). FSANZ has conducted a GM safety assessment for this product and has concluded that food derived from DHA canola is as safe for human consumption as food derived from non-GM canola cultivars. FSANZ's nutrition assessment concluded that consumption of DHA canola oil will not pose a nutritional concern to the Australian and New Zealand population.	Chapters 1 and 2	Noted. This information has been included in the RARMP.
9	Supports the conclusion that DIR 155 poses negligible risk of harm to human health and safety and the environment. However, would like to see more scientific rigour in the RARMP to support the Regulator's conclusion.	-	Noted. The Regulator's approach to risk analysis can be found in the Risk Analysis Framework at the OGTR website. The RARMP is based on the best available scientific evidence, further informed by input from experts, agencies and the Gene Technology Technical Advisory Committee.
	The RARMP suggests that DHA canola was approved for limited and controlled release in various LGAs in Victoria since 2014 and the Regulator has not received any report of adverse effects as a result of this release. However, limited and controlled trials are not designed to assess adverse or unexpected effects, especially in terms of toxicity or allergenicity to people or other	Chapters 1 and 2	Noted that field trials are not specifically designed to assess toxicity or allergenicity to people or other organisms. However, the licence holder is still obliged to report any adverse or unexpected effects to people dealing with the GMOs or other organisms in contact with the GMOs. The RARMP for this release considered information provided by the applicant as well as currently available scientific

Submission	Summary of issues raised	Consideration in RARMP	Comment
	animals. Suggests long term testing of the new proteins and any end products for possible toxic or allergenic responses in people, other organisms and the environment.		information from Australian and international sources. Chapter 1 of the RARMP discusses potential toxicity including the studies conducted on the introduced proteins and the end products. The RARMP concluded that risks to human health and the environment are negligible. FSANZ has conducted a safety assessment and concluded that food derived from the GM canola is safe for human consumption. The detailed assessment is available on FSANZ's website.
	Consideration for more research on the weediness and persistence of canola (including GM canola) in Australia to underpin future decision making.	-	The weediness of non-GM canola has been extensively studied both in Australia and overseas. This information is included in the canola biology document prepared by the Regulator - The Biology of *Brassica napus* L. (canola) and *Brassica juncea* (L.) Czern. & Coss. (Indian mustard), available from the OGTR website. The biology document is updated when new information from research becomes available and has been extensively used to inform the RARMPs for previously approved releases of GM canola.
10	Agrees with the overall conclusions of the RARMP.	-	Noted
	The Regulator should further consider the potential for pest species to gain a fitness advantage from consuming the GM canola.		Discussion of the potential for pest species to gain a fitness advantage has been expanded in Risk Scenario 3.

Appendix C Summary of submissions from the public on the consultation RARMP

The Regulator received one submission from the public on the consultation RARMP. The issues raised in this submission are summarised in the table below. All issues that related to risks to the health and safety of people and the environment were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Submission	Summary of issues raised	Consideration in RARMP	Comment
1*	Questions the integrity of the public servants who prepared the introductory blurb for this application, as it emphasises the beneficial side of altered omega-3 oil content of the GM canola but does not mention the presence of an herbicide resistance gene.	-	The introductory summary is circulated widely and invites experts, stakeholders and any member of the community to provide comment on the consultation RARMP. The purpose of the summary is to provide an introduction to the proposed release, including a brief description of the major traits that characterise the GMO(s); it directs the reader to the full RARMP for further information. The particular distinguishing trait for this application is that of modified omega-3 oil content. The full consultation RARMP for DIR 155, which is readily available on the OGTR webpage, provides more detailed information about the GM canola and includes a description of the introduced herbicide tolerance gene. The Question and Answer sheet published on that page also clearly discloses that, in addition to the seven introduced genes involved in fatty acid biosynthesis, the GM canola contains a selectable marker gene from a soil bacterium. As stated there, this gene confers tolerance to glufosinate herbicide, and was used during plant transformation to select for genetically modified plant cells in the laboratory.
	Has a number of concerns around horizontal gene transfer (HGT): The risk assessment considered the risk of vertical gene transfer (gene transfer to sexually compatible species), but did not consider the risk of HGT (gene transfer to sexually incompatible species). While this risk is reasonably low, the potential negative outcomes	Chapter 2	HGT was considered as a source of potential harm in Chapter 2, Section 2.2.2 of the RARMP. As outlined there, the potential for horizontal gene transfer from GMOs to other species that are not sexually compatible, and any possible adverse outcomes, has been reviewed in the scientific literature (Keese, 2008) as well as assessed in many previous RARMPs. Horizontal gene transfer was most recently considered in detail in the RARMP for DIR 108

Submission	Summary of issues raised	Consideration in RARMP	Comment
	could be severe. For example, our worst weed species could acquire one or more herbicide resistance genes and we could end up with more multi-herbicide resistance. A similar scenario has occurred with pathogenic bacterium, such as MRSA. Once this cultivar is released, it would be in the environment for a long time so HGT becomes more likely. This should be addressed in the assessment, even if only to provide legal protection against future litigation.		(canola). In previous assessments of horizontal gene transfer no substantive risk was identified, due to the rarity of these events and because similar gene sequences are already present in the environment and available for transfer via demonstrated natural mechanisms. For example, the introduced herbicide tolerance gene was isolated from the soil bacterium *Streptomyces viridochromogenes*, which is widespread in the environment. It is thus far more likely that, if HGT were to occur, it would be from naturally occurring *S. viridochromogenes* to weedy species rather than from the GM canola plants. This is different from the MRSA case as HGT between bacteria is far more likely to happen than HGT between plants and bacteria. Herbicide resistance in weeds is widely recognised as the result of adaptive evolution of weed populations to the selection pressure exerted by the targeting herbicides, rather than a sudden gain of any foreign herbicide resistance genes through HGT.

*This submission was originally sent to then Minister for Agriculture, Mr Barnaby Joyce, and his adviser, Mr Richard Hyett and received by the Department of Agriculture and Water Resources on 20 October 2017.