Australian Government
Department of Health
Office of the Gene Technology Regulator

September 2018

Risk Assessment and Risk Management Plan
for

DIR 163

Limited and controlled release of canola genetically modified for altered oil content and herbicide tolerance

Applicant - Nuseed Pty Ltd

PAGE INTENTIONALLY LEFT BLANK

Summary of the Risk Assessment and Risk Management Plan
for
Licence Application No. DIR 163

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application for the intentional release of a genetically modified organism (GMO) into the environment. A Risk Assessment and Risk Management Plan (RARMP) for this application was prepared by the Regulator in accordance with the 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 concluded that the field trial poses negligible risks to human health and safety and the environment and that any risks posed by the dealings can be managed by imposing conditions on the release.

The application

Application number	DIR 163
Applicant	Nuseed Pty Ltd (Nuseed)
Project title	Limited and controlled release of canola modified for altered oil content and herbicide tolerance
Parent organism	Canola (*Brassica napus* L.)
Introduced genes and modified traits	**Seven genes involved in the metabolism of long-chain polyunsaturated fatty acids:** • *Lackl-d12D* from the yeast *Lachancea kluyveri* • *Picpa- ω3D* from the yeast *Pichia pastoris* • *Micpu-d6D* from the microalga *Micromonas pusilla* • *Pyrco-d6E* from the microalga *Pyramimonas cordata* • *Pavsa-d5D* from the microalga *Pavlova salina* • *Pyrco-d5E* from the microalga *Pyramimonas cordata* • *Pavsa-d4D* from the microalga *Pavlova salina* **Four other genes for an altered oil profile**[1] **Two selectable marker genes:** • *pat* gene from the soil bacterium *Streptomyces viridochromogenes* for glufosinate tolerance. • *nptII* gene from *Escherichia coli* for kanamycin tolerance. **A herbicide tolerance gene**[2]
Proposed location	Site selection from 95 local government areas in New South Wales (NSW), Victoria (VIC) and Queensland (QLD).
Proposed release size	Up to 10 sites of 5 ha and 10 sites of 10 ha, i.e. up to 150 ha per year
Proposed release dates	Nov 2018 - December 2023

[1, 2] The identities of the genes have been declared as Confidential Commercial Information (CCI) under section 185 of the Act.

Primary purpose	To gather research and regulatory data, information and samples under field conditions for agronomic performance, oil profile and content, nutritional assessment, compositional analysis, molecular analysis, genetic stability and safety assessment.

Risk assessment

The risk assessment concludes that risks to the health and safety of people, or the environment, from the proposed release are negligible. No specific risk treatment measures are required to manage these negligible risks.

The risk assessment process considers how the genetic modification and proposed activities conducted with the GMOs 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 current scientific/technical knowledge, information in the application (including proposed limits and controls) and relevant previous approvals. Both the short and long term impacts are considered.

Pathways to potential harm that were considered included exposure of people or animals to the GM plant material, potential for persistence or dispersal of the GMOs, and transfer of the introduced genetic material to other non-GM canola, commercially approved GM canola plants or related species. Potential harms associated with these pathways included toxicity or allergenicity to people, toxicity to desirable animals, and environmental harms due to increased population size of animal pests and increased weediness.

The principal reasons for the conclusion of negligible risks are that the GM plant material will not be used for commercial human food or animal feed, and the proposed limits and controls effectively control the GMOs and their genetic material and minimise exposure.

Risk management plan

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the draft licence includes limits on the size, location and duration of the release, as well as controls to prohibit the use of GM plant material in commercial human food or animal feed, to minimise dispersal of the GMOs or GM pollen from the trial site, to transport the GMOs in accordance with the Regulator's guidelines, to destroy GMOs not required for testing or further planting, and to conduct post-harvest monitoring at each trial site to ensure the GMOs are destroyed.

Table of Contents

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*
DGLA	Dihomo-γ-linolenic acid
DIR	Dealings involving Intentional Release
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
FSANZ	Food Standards Australia New Zealand
GM(O)	Genetically modified (organism)
ha	Hectare
HGT	Horizontal gene transfer
km	Kilometre(s)
LA	Linoleic acid
LC-PUFA	Long chain polyunsaturated fatty acid
LGA	Local government area
m	Metre(s)
nptII	Neomycin phosphotransferase II
NSW	New South Wales
OA	Oleic acid
OGTR	Office of the Gene Technology Regulator
pat	phosphinothricin N-acetyltransferase
PC2	Physical containment level 2
QLD	Queensland
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*
VIC	Victoria
ω	Omega

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 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).

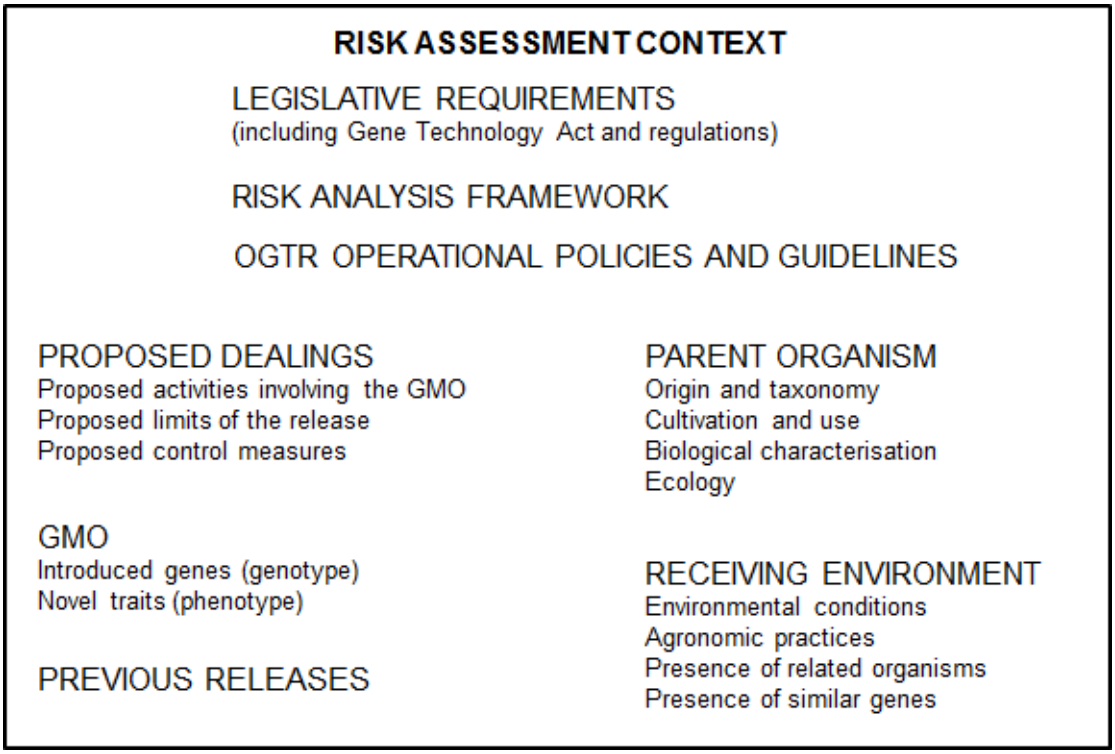

Figure 1. Summary of parameters used to establish the risk assessment context.

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, when 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. In accordance with section 50A of the Act, this application is considered to be a limited and controlled release application, as the Regulator was satisfied that: its principal purpose is to enable the applicant to conduct experiments and the applicant has proposed appropriate limits on the size,

location and duration of the release, as well as controls to restrict the spread and persistence of the GMOs and their genetic material in the environment. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

6. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee, Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, relevant local council(s), and the public. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. Five public submissions were received and they are summarised and addressed in Appendix B.

7. The *Risk Analysis Framework* (OGTR, 2013a) 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), the Australian Pesticides and Veterinary Medicines Authority, the 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 dealings

9. Nuseed proposes to release up to 80 lines of canola genetically modified for altered seed oil content and herbicide tolerance into the environment under limited and controlled conditions. The purpose of the release is to gather research and regulatory data, information and samples under field conditions for agronomic performance, oil profile and content, nutritional assessment, compositional analysis, molecular analysis, genetic stability and safety assessment of the GM canola.

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

- conducting experiments with the GMOs
- breeding the GMOs
- propagating the GMOs
- using the GMOs in the course of manufacture of a thing that is not a GMO
- growing the GMOs
- importing the GMOs
- transporting the GMOs
- disposing of the GMOs

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

3.1 The proposed limits of the dealings (duration, size, location and people)

11. The applicant proposes to conduct the trials on up to ten 5 ha sites and up to ten 10 ha sites, i.e. up to 150 ha per year, and over a maximum of five years from November 2018 to December 2023. The sites would be selected from 95 local government areas (LGAs) in NSW, VIC and QLD (Table 1). The selection of sites would depend on a number of factors, including: the availability of water and land during a growing season; adequate site distribution across Australian canola growing areas; the ability to ensure isolation and containment; and the ability to segregate from commercial canola crops. Details of site locations would be provided to the Regulator prior to each planting season.

Table 1. Proposed local government areas in which GM canola may be released.

New South Wales	Victoria	Queensland
Albury City Council	Ararat Rural City Council	Goondiwindi Regional Council
Balranald Shire Council	Ballarat City Council	Lockyer Valley Regional Council
Berrigan Shire Council	Benalla Rural City Council	Southern Downs Regional Council
Bland Shire Council	Greater Bendigo City Council	Toowoomba Regional Council
Blayney Shire Council	Buloke Shire Council	Western Downs Regional Council
Hilltops Council: Boorowa, Harden Shire and Young Shire Councils	Campaspe Shire Council	
Cabonne Shire Council	Central Goldfields Shire Council	
Carrathool Shire Council	Colac-Otway Shire Council	
Edward River Council: Conargo Shire and Deniliquin Councils	Corangamite Shire Council	
Coolamon Shire Council	Gannawarra Shire Council	
Coonamble Shire Council	Greater Geelong City Council	
Gundagai Council: Cootamundra and Gundagai Shire Councils	Glenelg Shire Council	
Federation Council: Corowa and Urana Shire Councils	Golden Plains Shire Council	
Cowra Shire Council	Hepburn Shire Council	
Forbes Shire Council	Hindmarsh Shire Council	
Gilgandra Shire Council	Horsham Rural City Council	
Griffith City Council	Indigo Shire Council	
Gunnedah Shire Council	Latrobe City Council	
Gwydir Shire Council	Loddon Shire Council	
Hay Shire Council	Macedon Ranges Shire Council	
Greater Hume Shire Council	Melton Shire Council	
Murrumbidgee Council: Murrumbidgee and Jerilderie Shire Councils	Mildura Rural City Council	
Junee Shire Council	Mitchell Shire Council	
Lachlan Shire Council	Moira Shire Council	
Leeton Shire Council	Moorabool Shire Council	
Liverpool Plains Shire Council	Mount Alexander Shire Council	
Lockhart Shire Council	Moyne Shire Council	
Mid-Western Regional Council	Murrindindi Shire Council	
Moree Plains Shire Council	Northern Grampians Shire Council	
Murray River Council: Murray Shire Council and The Council of the Shire of Wakool	Pyrenees Shire Council	

New South Wales	Victoria	Queensland
Muswellbrook Shire Council	Greater Shepparton City Council	
Narrabri Shire Council	South Gippsland Shire Council	
Narrandera Shire Council	Southern Grampians Shire Council	
Narromine Shire Council	Strathbogie Shire Council	
Orange City Council	Surf Coast Shire Council	
Parkes Shire Council	Swan Hill Rural City Council	
Tamworth Regional Council	Towong Shire Council	
Temora Shire Council	Wangaratta Rural City Council	
Snowy Valleys Council: Tumbarumba and Tumut Shire Councils	Warrnambool City Council	
Upper Hunter Shire Council	Wellington Shire Council	
Wagga Wagga City Council	West Wimmera Shire Council	
Walgett Shire Council	Wodonga City Council	
Warren Shire Council	Wyndham City Council	
Warrumbungle Shire Council	Yarriambiack Shire Council	
Weddin Shire Council		
Dubbo Regional Council: Dubbo City and Wellington Councils		

12. Only trained and authorised staff would be permitted to deal with the GM canola.

13. GM plant materials or products would not be used in commercial human food or animal feed.

14. Animal feeding experiments may be conducted in Australia or overseas. These could include acute oral toxicology, 90-day (rodent) feeding trials, broiler (chicken) feeding trials, aquaculture feeding trials and bioavailability (rodent) trials. These trials would only occur if Nuseed has all the appropriate approvals required for each trial. No animals from these studies would be allowed to enter the human food supply.

15. Other research with the oil may include sensory testing for feel, smell, taste and appearance and possible comparison with similar oils, to determine the acceptability of the oil alone or in food/feed offerings. Taste testing would be carried out in a similar way to wine tasting. Any nutritional study would be with highly refined oil containing omega-3 (ω-3) fatty acids. This would only occur with the appropriate oversight and protocols in accordance with the National Statement on Ethical Conduct in Human Research.

3.2 The proposed controls to restrict the spread and persistence of the GMOs in the environment

16. The applicant has proposed a number of control measures to restrict the spread and persistence of the GMOs and their introduced genetic material, each of which were considered in the evaluation of this application. These include:

- locating the proposed trial sites at least 50 m away from the nearest natural waterway
- restricting gene flow by controlling brassica weeds around the trial sites and adopting one of the following combination of controls (Figure 2):

a. cover the GMOs with Insect-proof tents from at least 7 days prior to flowering and until all GMOs have completed flowering, and surround the Planting Area with a 10 m Monitoring Zone and maintain a 400 m Isolation zone to other canola crops; or

b. surround the Planting Area with a 15 m Pollen trap of non-GM canola and a 50 m Monitoring Zone and maintain a 400 m Isolation Zone to other canola crops; or

c. surround the Planting Area with a 50 m Monitoring Zone and maintain an Isolation Zone of at least 1 km to other canola crops

- ensuring the Monitoring Zone is kept free of related species
- harvesting the GM canola separately from any other crops
- treating non-GM plants grown in the Planting Areas and Pollen Traps as if they were the GMOs
- cleaning any equipment used in connection with the GMOs as soon as practicable and before use for any other purpose
- transporting and storing GM plant material in accordance with the current Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs (2011)*
- destroying all plant material from the trial not required for further evaluation or future trials
- tilling the Planting Area and Pollen Trap, if used, partial buffer zone, any areas of land used to clean equipment and other areas where the GM material was dispersed within 60 days of harvest of the GMO to a maximum depth of 5 cm
- post-harvest monitoring the trial site at least once every 35 days for at least 2 years and until the site is free of volunteer plants for 12 months, and destroying any volunteer canola plants
- planting only crops permitted by the Regulator's (2013) Policy on Post- Harvest Crops permitted on GM Brassica trial sites during the post-harvest monitoring period.

17. Figure 2 shows the proposed site layout, including some of the controls. These controls, and the limits outlined above, have been taken into account in establishing the risk assessment context (this Chapter), and their suitability for containing the proposed release is reviewed in Chapter 3, Section 3.1).

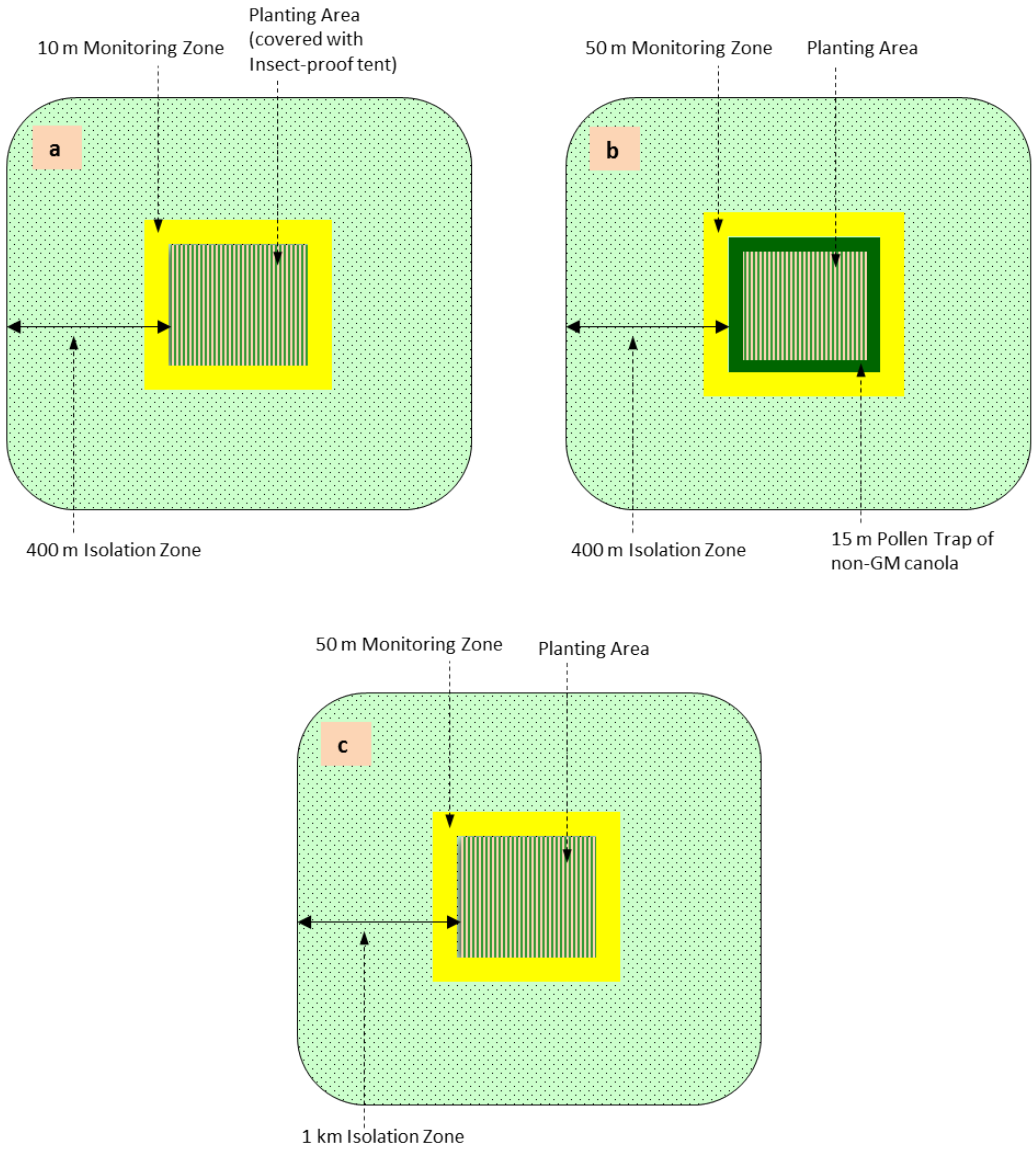

Figure 2. Proposed trial layout, including some of the controls (not to scale).
Site-layout (a) with Insect-proof tent, (b) without Insect-proof tent and with Pollen Trap, and (c) without Insect-proof tent or Pollen Trap. Monitoring and Isolation Zones would be kept free of intentionally grown related species.

Section 4 The parent organism

18. 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, NSW, VIC and South Australia. It is Australia's third largest broad acre crop (ABARES, 2018). 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 use of the parent organism in agriculture is summarised in Section 6 (the receiving environment).

19. The Standards Australia *National Post-Border Weed Risk Management Protocol* rates the weed risk potential of plants according to properties that 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). For canola, its actual rather than potential distribution is addressed. 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).

20. More detailed information regarding the parent organism can be found in the document *The Biology of Brassica napus L. (canola) and Brassica juncea (L.) Czern. & Coss. (Indian mustard)* (OGTR, 2017a), which was produced to inform the risk analysis process for licence applications involving GM canola plants and is available from the OGTR Biology Documents page. The proposed dealings with the GM canola are evaluated against non-GM canola and commercially approved GM canola as baselines.

Section 5 The GMOs, nature and effect of the genetic modification

21. The applicant proposes to grow up to 80 lines of GM canola with altered oil content, classified into four categories. Each line contains genes involved in creating an enhanced ω-3 oil profile. Some lines may also contain selectable marker genes or an introduced herbicide tolerance gene.

22. The genes introduced into category 1 GM canola are listed below (Section 5.2, Table 2).

23. GM canola with the same genetic material that was introduced into the category 1 lines was released in 2013 for field trial under licence DIR 123 and for commercial release in 2018 under licence DIR 155. Relevant information on the ω-3 fatty acid biosynthesis in brassicas is included in the RARMP for DIR 123 (OGTR, 2013b) and DIR 155 (OGTR, 2018a). This information is summarised and updated below.

24. Details of the identity of the regulatory elements for *nptII* expression as well as the genes introduced into categories 2, 3 and 4 of the GM canola, their source organisms, the specific traits they confer, the regulatory sequences and constructs used and other details have been declared Confidential Commercial Information (CCI) under section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

5.1 Long Chain-Polyunsaturated Fatty Acid biosynthesis

25. Fatty acids (FAs) consist of a hydrocarbon chain, saturated or unsaturated, capped by a carboxyl group (-COOH). FAs are infrequently present in cells as free molecules, and they are more commonly found as component of lipids, such as triacylglycerol (energy-storage lipids, also known as fats or oils) or phospholipids (lipid portion of cellular membranes) (Shahidi and Ambigaipalan, 2018). In plants, FAs are mainly synthesised *de novo* in plastids, and then transported to the endoplasmic reticulum for assimilation into specific lipid classes (Kim et al., 2013; Zhang et al., 2017).

26. Long-chain polyunsaturated fatty acids (LC-PUFAs) are fatty acids of 20 or more carbons in length with two or more cis double bonds in their backbone. Depending on the position of the first double bond counting from the methyl end of the FA, LC-PUFAs are divided into ω-6 (first double bond on the sixth carbon) and ω-3 (first double bond on the third carbon). Despite having vital roles in human health and development , mammals and plants only have part of the LC-PUFA biosynthetic pathway, and thus are unable to synthesise many of these LC-PUFAs *de novo* (Meesapyodsuk and Qiu, 2016). Unlike humans, plants can produce the precursor ALA that is present in some plant oils such as flaxseed, soybean, walnut and canola oils (Sharma, 2013). If humans consume ALA from these sources, the body is able to convert small amounts into other ω-3 LC-PUFAs. However, dietary supplementation is needed due to low conversion rates (Swanson et al., 2012). The complete LC-PUFA pathway mostly occurs in certain types of oceanic microorganisms, and, therefore, some of the important LC-PUFAs such as EPA and DHA only enter the human diet through oily fish and other marine sources (Ruiz-Lopez et al., 2015).

27. LC-PUFA synthesis can follow either the aerobic or the anaerobic biochemical pathway. The anaerobic pathway is highly complex and, so far, has not been introduced into GM plants.

28. The aerobic pathway consists of a series of desaturation (introduction of double bonds) and elongation (extension of carbon chains) steps of pre-existing FAs (Figure 3). This pathway occurs

mainly in animals and eukaryotic microorganisms (Sayanova and Napier, 2004). The enzymes involved have been isolated and characterised (Petrie and Singh, 2011). The aerobic pathway has been introduced into the GM canola proposed for release.

5.2 The genetic modifications in the GMOs proposed for release

29. Category 1 GM canola lines contain up to seven genes involved in the metabolism of the ω-3 LC-PUFA. Plants can produce the essential FAs ω-6 linoleic acid (LA) and ω-3 ALA (Figure 3). However, they lack the desaturases and elongases required for their conversion into other ω-3 LC-PUFAs, such as EPA, docosapentaenoic acid (DPA) and DHA (Ruiz-Lopez et al., 2012; Ruiz-Lopez et al., 2013). For the purpose of this document, these ω-3 LC-PUFA intermediates will be referred to as ω-3 LC-PUFAs or end products. The introduced genes convert the natural plant monounsaturated ω-9 FA oleic acid into ω-3 LC-PUFA products in the seed (Table 2 and Figure 3). The genes were sourced from yeast and marine microalgae, and codon optimised for expression in higher plants. They were synthesised *in-vitro* for transformation.

Table 2. LC-PUFA genes introduced into the category 1 GM canola.

Gene	Encoded protein	Source organism	Intended function*	Reference
Lackl-Δ12D	Δ12-desaturase	*Yeast* *Lachancea kluyveri*	Convert OA to LA	(Petrie et al., 2012)
Picpa-ω3D	Δ15−/ω-3 desaturase	Yeast *Pichia pastoris*	Convert LA to ALA	(Zhang et al., 2008)
Micpu-Δ6D	Δ6-desaturase	Microalgae *Micromonas pusilla*	Convert ALA to SDA	(Petrie et al., 2010b)
Pyrco-Δ6E	Δ6-elongase	Microalgae *Pyramimonas cordata*	Convert SDA to ETA	(Petrie et al., 2010a)
Pavsa-Δ5D	Δ5-desaturase	Microalgae *Pavlova salina*	Convert ETA to EPA	(Zhou et al., 2007)
Pyrco-Δ5E	Δ5-elongase	Microalgae *Pyramimonas cordata*	Convert EPA to DPA	(Petrie et al., 2010a)
Pavsa-Δ4D	Δ4-desaturase	Microalgae *Pavlova salina*	Convert DPA to DHA	(Zhou et al., 2007)

*****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 ($18:4^{\Delta 6,9,12,15}$).

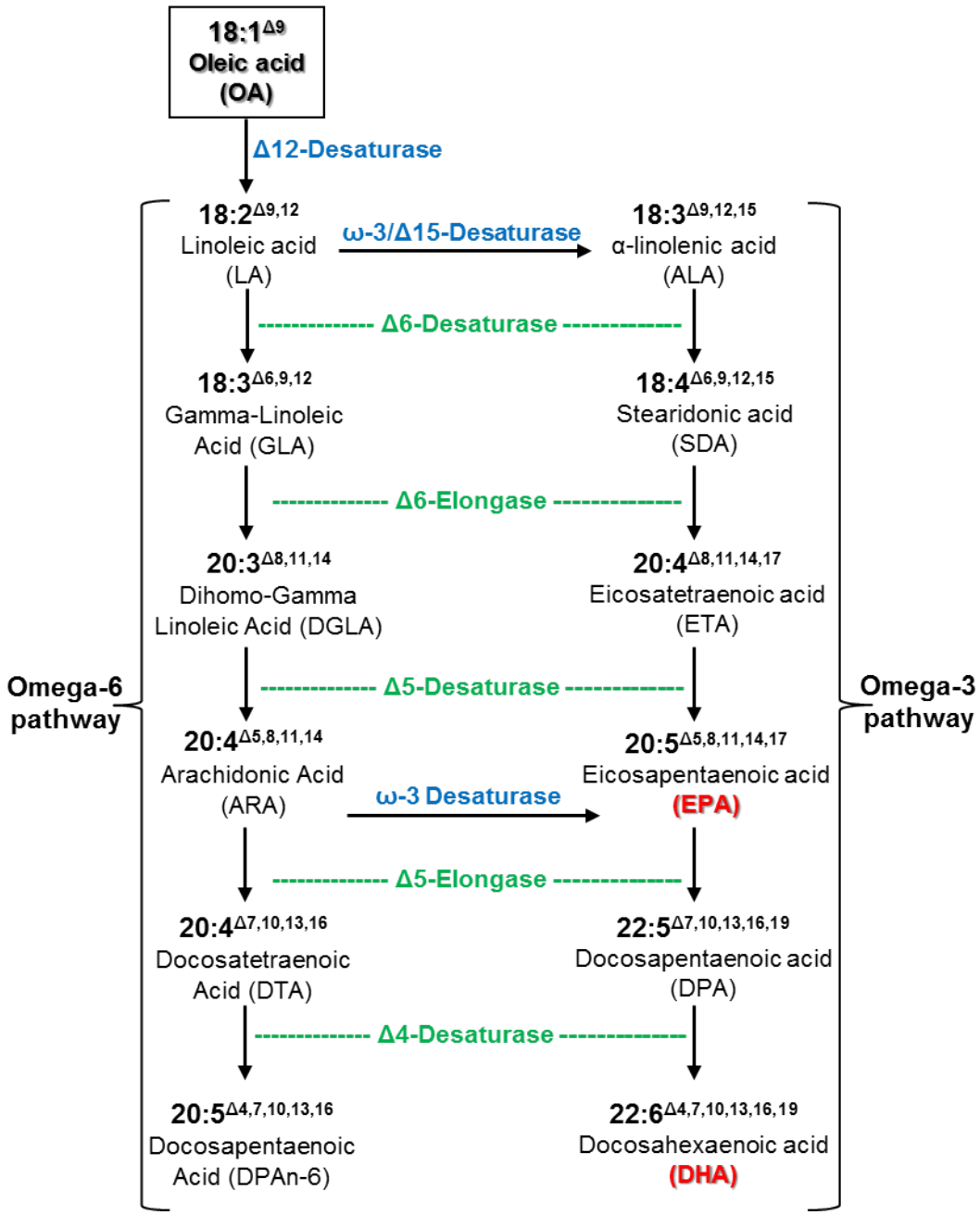

Figure 3. Outline of the ω-6 and ω-3 pathways for biosynthesis of LC-PUFA in GM canola, adapted from Ruiz-Lopez et al. (2013).
OA, LA and ALA are precursors commonly found in higher plants (Petrie & Singh 2011). ω-3 desaturases convert ω-6 FAs into their ω-3 counterpart, connecting both pathways (Ruiz-Lopez et al., 2015). Enzymes in blue are sourced from yeast, while enzymes in green originate from microalgae. The main ω-3 LC-PUFAs targeted for production are highlighted in red. The ω-3 desaturase from *Pichia pastoris* (also shown as ω-3/Δ15-desaturase) converts ω-6 FAs to the ω-3 form, and displays the same conversion rates to 18-carbon (LA) and 20-carbon (ARA) FAs (Zhang et al., 2008).

30. The applicant proposes to introduce other genes for an altered oil profile and a gene conferring herbicide tolerance to the other categories of GM canola.

31. The GM canola lines may also contain selectable marker genes used during initial development of the GM plants in the laboratory to select plant cells containing the introduced genes. The selectable marker genes are *nptII* and *pat. The nptII* gene codes for an aminoglycoside 3'-phosphotransferase II enzyme from *Escherichia coli*, also known as neomycin phosphotransferase II (NPTII), which inactivates aminoglycoside antibiotics such as kanamycin and neomycin. More information on *nptII* is available in the OGTR document *Marker genes in GM plants* (OGTR, 2017d). The *pat* gene codes for

phosphinothricin N-acetyltransferase (PAT) from *Streptomyces viridochromogenes*, and confers tolerance to glufosinate herbicides.

32. Short regulatory sequences that control gene expression are also present in the GM canola lines (Table 3). The expression of the genes introduced into category 1 GM canola is targeted to the seed with seed-specific promoters, while the expression of the selectable marker gene *pat* is driven by a constitutive promoter, which is active in all plant tissues. Other short regulatory sequences such as enhancers of gene expression and terminators were also used.

Table 3. Regulatory sequences present in the GM canola.

Sequence	Intended function	Source organism	Reference
Tobacco mosaic virus 5' UTR leader	Translational enhancer	Tobacco mosaic virus (TMV) 59	(Gallie et al., 1987)
MAR_Nicta- RB7	Rb7 matrix attachment region (MAR) for increasing gene expression	*Nicotiana tabacum*	(Hall et al., 1991; Halweg et al., 2005)
PRO_Arath- FAE1	Seed specific promoter	*FAE1* gene from *Arabidopsis thaliana*	(Rossak et al., 2001)
PRO_Brana-FP1	Seed specific promoter	*napA* gene from *Brassica napus*	(Stalberg et al., 1993)
PRO_Linus- Cnl1	Seed specific promoter	*conlinin1* gene from *Linum usitatissimum*	(Chaudhary et al., 2001)
PRO_Linus- Cnl2	Seed specific promoter	*conlinin2* gene from *L. usitatissimum*	(Chaudhary et al., 2001)
PRO_35S×2	Constitutive promoter	35S RNA gene from Cauliflower mosaic virus (CaMV)	(Kay et al., 1987; Coutu et al., 2007)
TER_Agrtu- NOS	Terminator	nopaline synthase *gene from Agrobacterium tumerfaciens*	(Bevan, 1984; Rogers et al., 1985; Sanders et al., 1987)
TER_Linus- Cnl1	Terminator	conlinin1 gene from *Linum usitatissimum*	(Chaudhary et al., 2001)
TER_Linus-Cnl2	Terminator	*Linum usitatissimum* conlinin2	(Chaudhary et al., 2001)
TER_Glyma-Lectin	Terminator	*Glycine max* lectin	(Vodkin et al., 1983; Cho et al., 1995)

33. Some of the GM canola lines would be produced using *Agrobacterium*-mediated transformation. This method has been widely used in Australia and overseas for introducing genes into plants. More information can be found in the document *Methods of Plant Genetic Modification* on the OGTR website (OGTR, 2018b). Some GM canola would be produced using other methods.

5.3 Toxicity/allergenicity of the proteins and end products associated with the introduced genes

34. Seven of the genes proposed for the current release, *Lackl-Δ12D*, *Picpa-ω3D*, *Micpu-Δ6D*, *Pyrco-Δ6E*, *Pavsa-Δ5D*, *Pyrco-Δ5E* and *Pavsa-Δ4D*, were present in GM canola approved previously for limited and controlled release under DIR 123 and for commercial release under DIR 155 (DHA canola) (see Section 7.1). These genes are involved in the biosynthesis of LC-PUFAs and the proteins encoded by the introduced genes are not expected to have any toxic or allergenic effects. An assessment of their toxicity and allergenicity was provided in the RARMPs for the previous approvals. No adverse effects have been reported for these releases. FSANZ has determined that food derived from DHA canola, which contains seven of the LC-PUFA genes proposed for the current release, is as safe for human consumption as food derived from conventional (non-GM) canola (FSANZ, 2017).

35. The end products, ω-3 LC-PUFAs, have been shown to promote health and help prevent disease in vertebrates (Carrie et al., 2002; McCann and Ames, 2005; Cutuli et al., 2014; Carragher et al., 2015; Twining et al., 2016). They are not known to be toxic to humans or other vertebrates, and they are common constituents of fish oils that form part of the normal human diet. Two long term human clinical trials showed no identifiable risks associated with 4 years consumption of doses of 10 or 30 mg of ω-3 DHA per kilogram of body weight per day (Wheaton et al., 2003; Hughbanks-Wheaton et al., 2014). However, cabbage white butterfly exposed to an experimental diet formulation supplemented with 1% canola oil (control) incrementally replaced with EPA and DHA (11:7 ratio) resulted in progressively heavier adults with an increased rate of wing deformities (Hixson et al., 2016).

36. The *pat* and *nptII* genes are commonly-used marker genes for selection of transformed plant cells, and neither introduced genes, nor the expressed proteins are new in the production of GM plants (Breyer et al., 2014). The *nptII* gene is derived from *E. coli*, a common gut bacterium that is widespread in human and animal digestive systems and ubiquitous in the environment. Further information about this gene can be found in the document *Marker genes in GM plants* (OGTR, 2017d). The *pat* gene is derived from *S. viridochromogenes*, a common soil bacterium that is not considered to be a pathogen of plants, humans, or other animals. Both genes have been used extensively and crops containing these genes have been assessed previously by the OGTR (most recently in RARMPs DIR 153 (*nptII*) and DIR 155 (*pat*)) and other regulatory agencies worldwide (OECD, 1999; EFSA, 2009; CERA, 2011; OGTR, 2017c, 2018a). GM foods containing the *nptII* and *pat* genes have been assessed and approved for use in food in Australia (FSANZ website) and worldwide. An extensive database exists regarding their safety and the scientific literature supports the conclusion that their presence in GM plants does not pose a risk to human health and safety, and that their encoded proteins are not toxic or allergenic.

5.4 Characterisation of the GMOs

37. The introduced genes are not known to confer any other phenotypic changes other than altered seed oil profile, antibiotic resistance and herbicide tolerance. Canola has previously been modified with similar genetic material and an altered ω-3 seed oil profile has been observed under DIR 123 (OGTR, 2013b) and DIR 155 (OGTR, 2018a). The applicant stated that observations of GM canola plants grown in PC2 glasshouses and plants grown in the field under DIR 123 do not indicate an unexpected phenotype. Some of the GM canola from each generation would be tested for the ω-3 phenotype in the seed using gas chromatography techniques.

38. The LC-PUFA pathway was introduced via *Agrobacterium*-mediated transformation. The applicant has not yet carried out a detailed molecular analysis for the presence or absence of vector or *Agrobacterium* sequences in the GM canola proposed for release. The GM plants would have been propagated by seed to at least the third generation from the transformation within PC2 glasshouses before the field trial, and *Agrobacterium* is not normally transmitted from one generation to the next via seed.

Section 6 The receiving environment

39. 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, 2013a).

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

6.1 Relevant abiotic factors

41. The proposed release would be carried out across a range of geographic and climatic conditions across Australia. The geographical distribution of commercial canola cultivation in Australia is limited by a number of abiotic factors, the most important being water availability. Germination of seed will only occur if there is sufficient soil moisture, and drought stress after anthesis can significantly reduce yield. Canola is also relatively sensitive to waterlogging which restricts root development (Walton et al., 1999; GRDC, 2009, 2017). Other abiotic stresses that can reduce canola yields include frost, particularly during early pod development, and heat stress (GRDC, 2009).

6.2 Relevant biotic factors

42. A number of diseases have the potential to significantly reduce the yield of canola. The fungal pathogen *Leptosphaeria maculans* causes blackleg, the most common and damaging disease affecting canola in Australia. Other serious diseases that affect canola production in Australia 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). These diseases are further discussed in the document *The Biology of Brassica napus L. (canola) and Brassica juncea (L.) Czern. & Coss. (Indian mustard)* (OGTR, 2017a).

43. 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).

44. 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 oats, volunteer cereals, and weeds from the *Brassicaceae* family. These were recently discussed in more detail in DIR 155 (OGTR, 2018a).

6.3 Relevant agricultural practices

45. Agronomic and crop management practices for the cultivation of the GM canola by the applicant would be the same as for commercial canola crops and would not differ from industry best practice used in Australia, except that the applicant proposes controls to minimise the dispersal and persistence of the GM canola (see Section 3).

46. Small areas/rows would be hand-planted or planted with a small plot seeder, larger areas with commercial equipment. In some instances drip/pipe irrigation, fertilisers, pesticides and other agronomic management practices may be used to maintain the crop. Standard cultivation and crop management practices for canola are discussed in DIR 155 (OGTR, 2018a) and in more detail in the documents *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).

6.4 Presence of related plants in the receiving environment

47. Canola is widely grown as an oil seed crop in Australia, and the proposed trial sites are located in commercial canola growing regions. Commercial canola in these areas includes non-GM canola and GM canola authorised for commercial release. Details of all GM canola varieties approved by the Regulator for commercial release in Australia are available from the OGTR website.

48. *Brassica napus* does not exhibit vegetative reproduction under field conditions, and it is predominantly self-pollinated. However cross-pollination occurs mainly through physical contact with neighbouring plants, and pollination can also occur via wind and insects. Outcrossing rates vary but average around 30% (Hüsken and Dietz-Pfeilstetter, 2007). The majority of small-scale release trials of GM canola revealed a dramatic decline in outcrossing rates when the distance from the GM source

increased (Funk et al., 2006). Outcrossing frequencies between adjacent fields are highest in the first 10 m of the recipient fields (Hüsken and Dietz-Pfeilstetter, 2007; OGTR, 2017a) with observations of most of the pollen dispersed within a 4.5 m area around the GM pollen source (Cai et al., 2008). However, low levels of GM canola pollen (less than 0.015%) have been measured up to 2 km from the source (Cai et al., 2008). 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, and gene flow between plants at 30 metre separation was reported to be 0.03% (Rieger et al., 2002).

49. Under natural conditions canola is receptive to outcrossing with sexually compatible crop species and with weedy relatives if there is synchronicity of flowering. More detailed discussion of *B. napus* hybridisation can be found in *The Biology of Brassica napus L. (canola) and Brassica juncea (L.) Czern. & Coss. (Indian mustard)* (OGTR, 2017a).

6.5 Presence of similar genetic elements, proteins and metabolic products in the environment

50. Some genes involved in ω-3 LC-PUFA biosynthesis are sourced from microalgae that are present in the marine environment (*P. salina*, *M. pusilla* and *P. cordata)*. The other genes in the category 1 GM canola were sourced from the yeasts *P. pastoris* and *L. kluyveri*, which are widely distributed in soil or on plants and fruits. All genes have been codon optimised for plants and *in-vitro* synthesised before transformation. This means that the DNA sequences may not occur naturally. However, the encoded proteins and end products are the same as those in the source organisms. Therefore, people naturally encounter the encoded proteins and metabolic products through contact with sea water, soil and plants, as well as consumption of certain fruits, nuts and seafood.

51. The *pat* gene was obtained from the common soil bacterium *S. viridochromogenes,* and it is a selectable marker that encodes for an acetyltransferase, a ubiquitous class of enzymes widely distributed in microorganisms, plants and animals. This gene is also present in other GM plants authorised for commercial release, including GM canola (DIR 021/2002, DIR 108 and DIR 155) and cotton (DIR 91). The RARMPs for these DIRs are available on the OGTR website. 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).

52. The *nptII* gene is derived from *E. coli*, a diverse group of bacteria part of the normal flora found in the intestines of warm-blooded organisms and widespread in the environment. This gene is also present in other GM plants authorised for commercial release, including GM cotton (DIR 066/2006, DIR 124, DIR 145), and canola (DIR 021/2002, DIR 108 and DIR 138). The RARMPs for these DIRs are available on the OGTR website.

53. Short regulatory sequences are derived from the bacterium *A. tumefaciens,* the plants *A. thaliana* (thale cress), *B. napus* (canola), *L. usitatissimum* (flax or linseed), *G. max* (soybean) and *N. tabacum* (tobacco), and the Cauliflower and Tobacco mosaic viruses. With the exception of tobacco, which is no longer grown commercially in Australia, all the source organisms for the introduced genetic elements are widespread and prevalent in the Australian environment and thus humans and other organisms would commonly encounter their genes, encoded proteins and regulatory sequences.

Section 7 Relevant Australian and international approvals

7.1 Australian approvals

Approvals by the Regulator

54. Some of the category 1 GM canola has been grown under DIR 123 (OGTR, 2013b). The GM canola in the other categories has not been released previously.

55. GM canola, safflower and cotton plants with an altered oil profile have been previously approved by the Regulator for both, limited and controlled as well as commercial release (see OGTR website for details). There are no reported adverse effects from these releases.

Approvals by other government agencies

56. The Regulator is responsible for assessing and managing 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.

57. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has assessed and approved the safety of food derived from DHA canola, which contains seven of the LC-PUFA genes proposed for the current release. FSANZ has determined that food derived from DHA canola is as safe for human consumption as food derived from conventional (non-GM) canola (FSANZ, 2017). The applicant does not intend to use materials from this trial in commercial human food and, accordingly, no application has been submitted to FSANZ. FSANZ approval would need to be obtained before materials from these GM canola lines could be sold as food.

58. The APVMA has regulatory responsibility for agricultural chemicals, including herbicides, in Australia. If the applicant intends to apply herbicide to the GM canola during the trial, this may be subject to regulation by the APVMA.

59. GM canola seed may be imported into Australia, which may require an import permit from the Department of Agriculture and Water Resources.

7.2 International approvals

60. Nuseed has obtained permits to conduct research trials of DHA canola in Canada and the United States of America (USA) since 2016. DHA canola contains seven of the LC-PUFA genes in the current application, and is the trade name for the GM canola approved by the regulator under DIR 155. Trials were carried out over two years in Canada and the USA.

61. Applications for DHA canola were made in 2017 to the relevant authorities in USA (Food and Drug Administration for food and feed, and United States Department of Agriculture Biotechnology Regulatory Services for environment) and Canada (Health Canada for food, and Canadian Food Inspection Agency for environment). The US Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) deregulated DHA canola in August 2018.

Chapter 2 Risk assessment

Section 1 Introduction

62. 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 4).

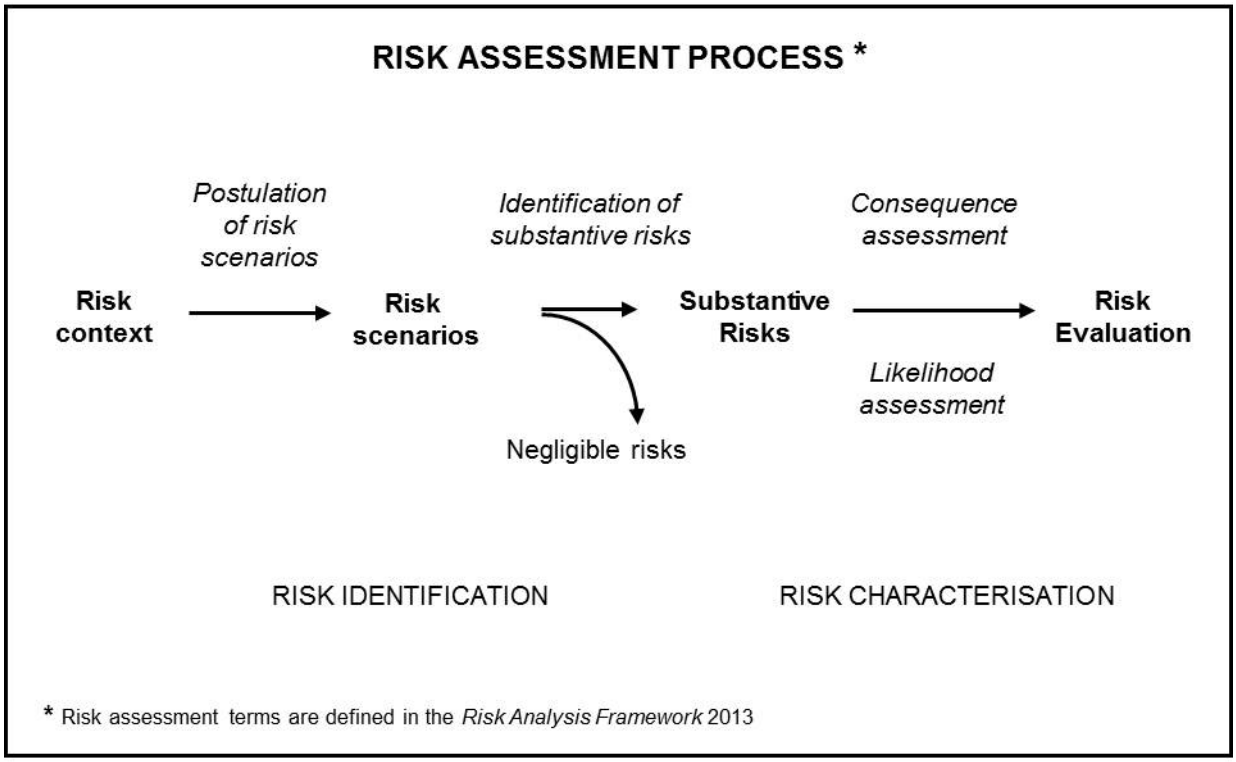

Figure 4. The risk assessment process.

63. 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.

64. 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.

65. 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, 2013a). 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 used to postulate risk scenarios (Keese et al., 2014). Risk scenarios postulated in previous RARMPs prepared for licence applications of the same or similar GMOs are also considered.

66. Substantive risks (i.e. 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

67. 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).

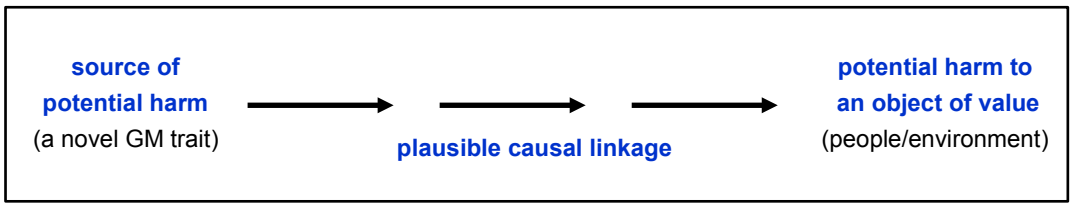

Figure 5. Components of a risk scenario.

68. In addition, the following factors are taken into account when postulating relevant risk scenarios:

- 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

69. 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 genetic elements

70. Category 1 GM canola has been modified by the introduction of up to seven genes involved in a novel ω-3 LC-PUFA production pathway in the seed. The introduced genes, their encoded proteins and the metabolic end products of the ω-3 LC-PUFA pathway were considered as a potential source of risk in canola in DIR 123 (OGTR, 2013b) and DIR 155 (OGTR, 2018a), and will be considered again here.

71. In addition, four other genes for an altered ω-3 seed oil profile have been introduced in other categories of GM canola. The effects of the introduced genes, encoded proteins and end products will be considered.

72. Some of the GM canola lines contain the selectable marker genes *nptII* and/or *pat*. 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, 2016). Similarly, the *nptII* gene is present in GM canola plants authorised under DIR 021/2002, DIR 108 and DIR 138. These genes and their products have already been extensively characterised and assessed as posing negligible risk to human and animal health and to the environment as a result of gene technology by regulatory agencies in Australia and overseas. Therefore they will be not considered further for this application.

73. The herbicide tolerance gene has been declared commercial confidential information (CCI) under Section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

74. The herbicide tolerance gene is not part of the ω-3 LC-PUFA biosynthesis pathway and there is no evidence that the enzymes would interact in the GM canola to produce any new products or to generate any new risks. Therefore the combination of the oil pathway genes with the herbicide tolerance gene will not be considered further for this application.

75. The introduced genes are controlled by regulatory sequences. These regulatory sequences are derived from common plants, a bacterium and plant viruses (see Table 3). Regulatory sequences are naturally present in plants, and the introduced elements are expected to operate in similar ways to endogenous elements. Although the *A. tumefaciens* and the Tobacco and Cauliflower Mosaic Virus are plant pathogens, regulatory sequences are not expressed as proteins and dietary DNA has no toxicity (Society of Toxicology, 2003). Regulatory sequences have no pathogenic, toxic or carcinogenic properties, and cannot of themselves cause disease. Hence, risks from the use of the introduced regulatory elements themselves will not be considered further for this application.

Unintended effects

76. 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 use, with few documented cases where conventional breeding has resulted in an unacceptable level of a metabolite in a crop (Berkley et al., 1986; Seligman et al., 1987), and no documented reports of conventional breeding leading to the production of a novel toxin or allergen (Steiner et al., 2013). Current practices identify and remove harmful non-GM plants to protect domesticated animals and people (Steiner et al., 2013). Therefore, unintended effects resulting from the process of genetic modification will not be considered further.

2.2 Causal pathway

77. 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), gene product(s) and end products
- potential exposure to the introduced gene(s), gene product(s) and end products 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 GMOs, (e.g. reproductive characteristics, dispersal pathways and establishment potential)
- tolerance to abiotic conditions (e.g. climate, soil and rainfall patterns)
- tolerance to biotic stressors (e.g. pest, pathogens and weeds)
- tolerance to cultivation management practices
- gene transfer to sexually compatible organisms
- gene transfer by horizontal gene transfer (HGT) and
- unauthorised activities.

78. 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.

79. The potential for horizontal gene transfer (HGT) and any possible adverse outcomes has been reviewed in the literature (Keese 2008) and assessed in previous RARMPs. HGT was most recently considered in the RARMP for DIR 108 (OGTR, 2011). HGT events rarely occur and the wild-type gene sequences are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, risks from HGT will not be assessed further.

80. Previous RARMPs have considered the potential for unauthorised activities to lead to an adverse outcome. The Act provides 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, risks from unauthorised activities will not be considered further.

2.3 Potential harm

81. Potential harms from GM plants include:

- harm to the health of people or desirable organisms, including toxicity/allergenicity
- reduced biodiversity through harm to other organisms or ecosystems
- 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 (e.g. providing food or shelter for pests or pathogens) or abiotic environment (e.g. negative effects on fire regimes, nutrient levels, soil salinity, soil stability or soil water table).

82. These harms are based on those used to assess risk from weeds (Keese et al. 2013; Standards Australia Ltd et al. 2006). Judgements of what is considered harm depend on the management objectives of the land where the GM plant may be present. 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

83. Four risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 4, and discussed individually below. 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 a substantive risk.

Table 4. Summary of risk scenarios from the proposed dealings with GM canola.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	GM canola expressing the introduced genes for an altered ω-3 seed oil profile	Cultivation of GM canola at trial sites ⬇ Exposure of people and other desirable organisms to GM canola encoded proteins or end products	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms	No	• The proteins encoded by the introduced genes occur naturally in the environment and are not known to be toxic or allergenic to people or toxic to other organisms. • The ω-3 LC-PUFAs and the ω-3 seed oil are not known to be toxic to humans or other vertebrates. • The GM canola would not be used in commercial human food or animal feed. • The limited scale, and other proposed limits and controls minimise exposure of people and other organisms to the GM plants.
2	GM canola expressing the introduced genes for an altered ω-3 seed oil profile	Cultivation of GM canola at trial sites ⬇ GM canola seed ingested by pests ⬇ Increased fitness of pests	Reduced establishment or yield of desirable plants OR Reduced biodiversity	No	• The limited scale and other proposed limits and controls minimise exposure of pests to the GM seeds. • Pests are controlled by current pest management practices.
3	GM canola expressing the introduced genes for an altered ω-3 seed oil profile	Cultivation of GM canola at trial sites ⬇ Dispersal of GM seed outside trial limits ⬇ Establishment of populations of volunteer GM plants expressing the introduced genes in the environment	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable plants	No	• Canola is not a persistent weed in agricultural areas or intensive use areas or nature reserves. • The limited scale and other proposed limits and controls minimise the spread and persistence of the GM canola seeds outside the trial limits. • Weed management strategies, including the use of herbicides, can control volunteer GM canola. • Risk scenario 1 did not identify toxicity or allergenicity of the GMOs as a substantive risk.
4	GM canola expressing the introduced genes for an altered ω-3 seed oil profile	Cultivation of GM canola at trial sites ⬇ GM canola pollen flow outside the trial site ⬇ Outcrossing with other sexually compatible plants ⬇ Establishment of populations of hybrid GM plants expressing the introduced genes in the environment	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable plants	No	• The proposed limits and controls would minimise pollen flow to sexually compatible plants outside the trial sites. • Risk scenarios 1 and 3 did not identify toxicity, allergenicity or weediness of the GMOs as substantive risks. Risks associated with the GM hybrids are unlikely to differ to those posed by GM canola plants.

Risk scenario 1

Risk source	GM canola expressing the introduced genes for an altered ω-3 seed oil profile
Causal pathway	⬇ Cultivation of GM canola at trial sites ⬇ Exposure of people and other desirable organisms to GM canola encoded proteins or end products ⬇
Potential harm	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms

Risk source

84. The source of potential harm for this risk scenario is GM canola expressing the introduced genes for an altered ω-3 seed oil profile.

Causal pathway

85. GM canola would be cultivated at trial sites and the introduced genes for an altered seed oil profile expressed. People and other desirable organisms could be exposed to the introduced genes and end products.

Exposure of people

86. Authorised and trained personnel would be exposed to the GM canola and plant material while cultivating, harvesting, transporting, experimenting or conducting other dealings with GM canola. Exposure of workers is possible by dermal contact and inhalation.

87. Another potential route of exposure to the introduced proteins and end products is ingestion. Limited sensory oil tasting would be carried out subject to oversight by a Human Research Ethics Committee which is required to review and approve the research proposals in accordance with the National Statement on Ethical Conduct in Human Research. Oil testing would be similar to wine testing and most of the oil tested would not be consumed. Canola oil is highly refined and does not contain detectable amounts of protein. Therefore, any exposure would only be to the introduced end products. GM canola would not be used in commercial human food and, therefore, the public would not be exposed to the GM canola oil.

88. Because of the proposed limits and controls, there is little potential for the public to be exposed to the GM canola.

Exposure of other desirable organisms

89. A number of other desirable organisms may also be directly exposed to the introduced proteins and end products through ingesting the GM canola (such as livestock, wild animals, birds or insects). Livestock would not be expected to ingest the introduced proteins as the GM plant material is not to be used in commercial animal feed. No grazing of livestock would be permitted in the planting area or pollen trap during the trial or after the trial until the Regulator was satisfied that no GMOs remained at the trial site. Feral pigs or kangaroos are difficult to exclude from fields. Rodents, especially mice, and birds might also feed on GM canola. Some animals may be fed non-viable material from the GMOs under approved experimental conditions.

90. Soil organisms, such as earthworms, might come into contact with decomposing GM canola. Also, pollinators such as honeybees would be exposed to nectar and pollen from the GM canola, as they commonly use canola as a source of nectar and pollen. However, the expression of the introduced genes to alter oil content is targeted to the seed (see Chapter 1, Section 5.2). Therefore, pollinators would have minimal or no exposure to these proteins, or to other pathway products, through nectar and pollen. The proposed isolation measures to limit gene flow through pollen movement will also minimise the likelihood of GM canola pollen occurring in honey from nearby hives.

Furthermore, commercial procedures used for honey processing (e.g. sieving and filtering) will reduce the presence of GM canola pollen in honey (reviewed in RARMP for DIR 123, (OGTR, 2013b)).

91. At the end of the trial, the applicant proposes to destroy GM canola not required for further research purposes. The proposed limits and controls would restrict the potential for exposure to the GM canola.

Potential harm

92. People and other desirable organisms exposed to the introduced genes, proteins and end products may show increased toxic or allergic reactions compared to those exposed to non-GM canola or commercially approved GM canola.

93. Allergenicity is defined as the potential of a substance to cause an immunological reaction following its ingestion, dermal contact or inhalation, which may lead to tissue inflammation and organ dysfunction (Arts et al., 2006). 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). The introduced genes could lead to increased toxicity or allergenicity of the GM canola for people or increased toxicity to other desirable organisms.

Toxicity and allergenicity to humans and toxicity to other vertebrates

94. Occupational exposures to canola pollen, dust and flour have been implicated in allergic reactions in people and a number of putative allergens have been characterised, including seed storage proteins. Since the introduced genes are only expressed in seeds, GM canola pollen and dust are not expected to have increased allergenicity or toxicity compared to non-GM or commercially approved GM canola pollen. Bioinformatic analyses have not found sequence identity or immunological relevant similarities between the introduced genes and encoded proteins approved in DIR 123 and known allergens. Handling of GM canola by authorised staff has not resulted in any adverse health effects. As discussed in Chapter 1, section 6.5, the introduced genes and the encoded proteins are the same or similar to those present in organisms that are present in the environment and are not known to be toxic to humans or animals. People and animals are likely to naturally encounter the introduced genes and encoded proteins through contact with sea water, soil and plants, as well as consumption of certain fruits, nuts and seafood, such as fish and shellfish. Therefore, it is not expected that they will be toxic or allergenic, or lead to increased toxicity or allergenicity.

95. The introduced genes specifically confer an altered ω-3 oil profile in the GM canola seed. Consequently, GM canola oil may contain various levels of ω-3 LC-PUFAs end products, which are not naturally produced in non-GM canola. These end products are not known to be toxic to humans or other vertebrates, and they are common constituents of fish oils that form part of the normal human diet. Numerous studies have revealed the benefits of DHA and other ω-3 LC-PUFAs to human health, including cardiovascular diseases, diabetes, cancer, mental illnesses and others (Whelan, 2009; Byelashov et al., 2015; Dyall, 2015; Huang et al., 2016; Shahidi and Ambigaipalan, 2018). Two long term human clinical trials showed no identifiable risks associated with 4 years consumption of high doses of the ω-3 DHA (Wheaton et al., 2003; Hughbanks-Wheaton et al., 2014). No allergic reactions to canola oil have been reported in people (Gylling, 2006; OGTR, 2017a). Some cases of food allergy to rapeseed have been reported, but these are rare (Fiocchi et al., 2016). Therefore, the GM canola seed oil with an altered ω-3 profile is not expected to lead to increased toxicity or allergenicity.

96. FSANZ approved DHA GM canola and determined that food derived from the GM canola is as safe for human consumption as food derived from non-GM canola varieties (FSANZ, 2017).

97. GM canola plants with introduced genes involved in the biosynthesis of ω-3 LC-PUFAs have been previously approved by the Regulator. To date, no adverse effects have been reported to the Regulator from these releases.

98. Studies on vertebrate species have not identified a potential for toxicity of ω-3 LC-PUFAs (see discussion in Risk scenario 2).

Toxicity to other organisms

99. A study reported that adult individuals of the pest cabbage white butterfly (*Pieris rapae*) were heavier and had an increased rate of wing deformities as a result of ω-3 LC-PUFAs consumption (Hixson et al., 2016). However, white butterfly caterpillars feed on leaves, and the altered oils in the GM canolas reviewed under this application is confined to the seeds. Consequently, there is some uncertainty for this risk assessment regarding the potential negative effects that the consumption of GM canola could have in desirable arthropods.

Conclusion

100. Risk scenario 1 is not identified as a substantive risk due to limited exposure and lack of known toxicity or allergenicity of the introduced genes, encoded proteins and end products to humans and other vertebrates. Also, the GM plant material would not be used as livestock feed, and other proposed limits and controls would restrict exposure of people and animals to the GM plant material. Therefore, this risk could not be greater than negligible and does not warrant further assessment.

Risk scenario 2

Risk source	GM canola expressing the introduced genes for an altered ω-3 seed oil profile
Causal pathway	⬇ Cultivation of GM canola at trial sites ⬇ GM canola seed ingested by pests ⬇ Increased fitness of pests ⬇
Potential harm	Reduced establishment or yield of desirable plants OR Reduced biodiversity

Risk source

101. The source of potential harm for this postulated risk scenario is GM canola expressing the introduced genes for an altered ω-3 seed oil profile.

Causal pathway

102. The GM canola would be cultivated at trial sites and the introduced genes for an altered seed oil profile expressed. Pests may ingest the GM canola seed and have an advantage when compared with pests eating non-GM or commercially approved GM canola seeds. The populations of these pests may then increase rapidly as a consequence of this advantage.

Exposure of pest animals to the GM canola seed with altered oil profile

103. The most prevalent arthropod pests during flowering and maturation on canola in Australia are mainly non-native and include the cabbage aphid (*Brevicoryne brassicae*), turnip aphid (*Lipaphis erysimi*), green peach aphid (*Myzus persicae*), cowpea aphid (*Aphis craccivora*), diamondback moth (*Plutella xylostella*), heliothis caterpillars (*Helicoverpa armigera* and *H. punctigera*), cabbage centre grub (*Hellula hydralis*), cabbage white butterfly (*Pieris rapae*), looper caterpillars (*Chrysodeixis* spp.), seed bugs (family Lygaeidae), Rutherglen bug (*Nysius vinitor*), grey cluster bug (*Nysius clevelandensis*) and plague thrips (*Thrips imaginis*).

104. The most important vertebrate pests eating developing and mature seed are mice. Mice are well-known to eat developing and mature seeds, including seed pods as well as seed after sowing and young seedlings (GRDC, 2011).

105. These recognised arthropod and vertebrate pests may be exposed to the GM canola during the proposed field trial.

106. Animals, such as kangaroos, feral pigs or emus may occasionally eat canola. Similarly, other Australian native birds, including little corellas (*Cacatua sanguinea*), galahs (*Eolophus roseicapilla*), cockatiels (*Nymphicus hollandicus*), crested pigeons (*Ocyphaps lophotes*), wood ducks (*Chenonetta jubata*) and Australian ringnecks (*Barnardius zonarius*) may cause some damage by feeding on developing or mature seeds of canola or by scratching soil into which seeds have recently been sown (Bomford and Sinclair, 2002; Tracey et al., 2007; Jackson, 2009; Latitude_42, 2011). Non-native birds, such as house sparrows (*Passer domesticus*), may visit canola crops (Department of Primary Industries website, accessed in May 2018). Quails (Galliformes), doves (Columbidae), finches (Fringillidae), and juncos (*Junco spp*.) have been listed as eating rapeseed (All about birds website, accessed in May 2018).

107. There are no control measures used for birds in canola in Australia (Australian Oilseeds website, accessed in May 2018). This could either be because canola growers do not consider it necessary to control birds or because there simply is no effective control for pest birds in broad acre crops. Considering that canola is the third largest broad acre crop in Australia (OGTR, 2017a), the former is considered more likely.

108. Although recognised canola pests such as mice, kangaroos and feral pigs would be present in canola growing areas and might cause localised damage in canola fields, the potential for them to be exposed to the GM canola proposed for release would be unlikely compared to their exposure to non-GM and commercially approved GM canola, due to the small size of the trial. In Australia, the area planted to canola was approximately 2.3 million ha per year over the last two years, with areas in NSW and VIC combining to just below 1.0 million ha (AOF, 2017). The proposed GM trial area of 150 ha would represent approximately 0.015% of the canola growing area in those two states.

109. In QLD, canola is only grown sporadically and it is suited to the southern subtropical zones, where is grown as a winter crop (OGTR, 2017a)Agrifutures website, accessed in August 2018). This would suggest that pests which are specific to canola would not be expected to populate these areas to a great degree, whereas pests which are non-specific, including some of the arthropods above as well as mice, may occur in the QLD areas proposed for release. The latter pests may be exposed to the GM canola seeds. These pests would not be used to canola as a food source, and they would also have other food sources available in the same planting areas. An example of an alternative food source is wheat, the major winter crop in QLD, with an estimated 670,000 ha to be planted in 2018-2019 in Southern and Central QLD (Department of Agriculture and Fisheries Queensland, accessed in August 2018, ABARES website, accessed in August 2018). Chickpeas and barley are commonly grown crops (250,000 and 155,000 ha respectively). These crops are important food sources for pests. Other important winter crops in this region that pests have access to are oats, cereal rye, lupins, field peas, faba beans, vetch, lentils and safflower.

110. Pests could be exposed to the GM canola seed expressing the introduced proteins and the altered oil profile prior to seed germination, while seed develops and matures, and while mature seed is available in the field. This would give canola pests a small window during each of the five years within which they may be exposed to the GM canola seed. However, in agricultural cropping areas, food is not limiting for pests, that already have access to other sources of ω-3 FA such as aquatic insects, some seeds, leaves and nuts (Twining et al., 2016). Therefore, short term intermittent exposure of pests to the GM canola seeds is not expected to lead to sustained benefits to many pest species.

111. Additionally, as part of crop management, farmers are recommended to scout for these pests regularly in-crop or on-farm, and apply control measures to reduce their numbers before serious infestations ensue.

112. For this proposed field trial, pests are not expected to have access to the GM canola seed during storage as it would occur under the current Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs (2011).*

Potential of pests to have an advantage when ingesting GM canola seed with an altered seed oil profile

113. Few studies have investigated the potential of ω-3 LC PUFAs to affect recognised canola pests. This is an area of uncertainty for this risk assessment.

114. For invertebrate pests, a study reported negative effects on the pest cabbage white butterfly associated with ω-3 LC-PUFA consumption. Adult butterflies that originated from larvae fed with EPA and DHA were heavier and had an increased rate of wing deformities compared to the control group (Hixson et al., 2016).

115. In vertebrate species, the ingestion of DHA or other ω-3 LC-PUFAs may have a beneficial effect on their general health (Carrie et al., 2002; McCann and Ames, 2005; Cutuli et al., 2014; Carragher et al., 2015; Twining et al., 2016). Some vertebrate canola pests might not usually have access to the introduced ω-3 LC-PUFAs as part of their diets. Thus, it is possible that the accessibility of these health enhancing FAs to (terrestrial) vertebrate pests could lead to an increase in their populations (Colombo et al., 2018).

116. The consumption of oil enriched with ω-3 LC-PUFAs has been associated with a number of health benefits in humans and some animals. A study performed in food-limited American tree swallows which live in areas where they naturally have access to ω-3 FAs revealed benefits linked with ω-3 LC-PUFAs availability: a higher amount of EPA and DHA contributed to the development of healthier chicks (Twining et al., 2016).

117. Animal studies linking dietary ω-3 LC-PUFAs with male reproductive capacity have yielded variable and often contradictory results. While some studies found that the changes in the lipid composition of semen caused by dietary ω-3 LC-PUFA supplementation led to positive effects on the efficiency of male reproductive system of rams, bulls and boars (Alizadeh et al., 2014; Khoshvaght et al., 2016; Lin et al., 2016), other reports suggest that the changes observed in sperm lipid composition did not resulted in improvements of many of the semen quality parameters in rams (Fair et al., 2014), young post-pubertal dairy bulls (Byrne et al., 2017), boars (Castellano et al., 2010) or rabbits (Gliozzi et al., 2009). In laying hens and cockerels, fish oil treatment resulted in the lowest egg weights and fertility, but higher hatchability (Olubowale et al., 2014). The reproductive capacity of older male turkeys was sustained longer when their diet contained a higher ratio of ω-3/ω-6 PUFAs (Blesbois et al., 2004). In chickens, both ω-3 and ω -6 rich diets showed an age-dependent positive effect on fertility (Cerolini et al., 2003). Considering these positive, neutral and negative effects on fertility in animals, this is an area of uncertainty for this risk scenario.

118. The contribution of these FAs to the reproductive fitness of other short-lived vertebrate pest species, such as mice and rats, has not been demonstrated. A study in young rats showed that, despite the positive effects of dietary DHA for maintaining the functions of important organs, this could not be linked to increased fitness or competitiveness enhancement (DeMar et al., 2008). Another study in rats showed that dietary vitamin E is more effective in improving rat sperm than ω-3 and ω-6 PUFAs (Alizadeh et al., 2016).

119. It is also possible that terrestrial animals that have evolved without sustained access to marine sources of ω-3 LC-PUFAs have fewer limitations in their synthesis from precursors, and therefore have less dependence on external sources of ω-3 LC-PUFAs (Martinez Del Rio and McWilliams, 2016). This is illustrated by a study in mice where the lipid biosynthesis pathway was intentionally disrupted. As a result, the concentration of brain DHA was reduced independent of the mice diet, and mice developed multiple abnormalities such as male infertility and eye development problems (Rodemer et al., 2003).

120. Effects on semen of birds and mammals are not only related to consumption of ω-3 LC-PUFAs from GM canola. DHA and other ω-3 and ω-6 LC-PUFAs, oleic and linolenic acid are found in flaxseed, soybean, fish and sunflower, and they also have the potential to alter fertility rates, sexual behaviour and semen characteristics (Zubair and Khalique, 2016). In rodents, supplementation with PUFA from sunflower and olives has been shown to disrupt sperm (Cardoso et al., 2014).

121. There have been no reports of increased pest activities from previous limited and controlled releases of GM canola with altered oil content or from releases of other GM crops with similar or different changes to the seed oil profile, noting that some of those other GM crop releases required specific controls, such as bird scarers.

Potential of pest populations to increase to greater numbers than pests fed other canola

122. In nature, the population sizes of many animal species increases and decreases over time, depending on both biotic (e.g. limitation and quality of food, predation) and abiotic conditions (e.g. environmental conditions and extreme weather events such as fires and floods) (Nature knowledge Project website, accessed in May 2018).

123. Severe infestations of canola crops with invertebrate pests have been reported. For example, cabbage aphid, turnip aphid, green peach aphid and cowpea aphid have led to canola yield losses of up to 33% (GRDC, 2009). Birds are also known to lead to some localised damage in broad acre crops, including canola (Bomford and Sinclair, 2002; Tracey et al., 2007; Jackson, 2009), and mouse plagues have an adverse effect in both agricultural and natural areas (GRDC, 2011). However, recently a more sustained population of mice in agricultural areas has been observed (GRDC website, accessed in May 2018).

124. Generally, pest populations increase rapidly when abiotic and biotic environmental conditions are favourable. The trials are proposed for canola growing areas. Food would already be abundant during the field trial. Therefore, the proposed field trial would only contribute to food availability and nutritional benefit on limited areas over the limited time during which GM canola seeds are available, and only for those pest species that may gain an advantage by eating the GM canola seeds.

125. Pest populations are known to decrease rapidly when either biotic or abiotic environmental conditions change and become unfavourable for the pest. For example, populations of predators of a pest may increase, food or water may become scarce, temperatures may become unfavourable for the pest or people may use an effective control method. With the exception of a possible nutritional benefit on limited areas over the limited time during which GM canola seeds are available, these factors would not be influenced by the proposed field trial.

126. The proposed limits, i.e. the size and duration, and controls such as maintaining a well-managed monitoring zone around the planting areas or pollen traps, if used, are expected to limit exposure of pest animals to the GM canola proposed for release. In addition, other canola would be available as food for the canola pests as the release is proposed to take place in canola growing areas. Also, if a licence were issued, the licence holder would be required to report any unintended effects from the authorised dealings to the Regulator.

Potential harm

127. If pests consuming the GM canola seeds with an altered oil profile had an advantage over those eating non-GM canola or commercially approved GM canola, and populations increased to a greater extent than under current conditions, they may have a greater negative effect on native or other desirable vegetation, and could reduce the establishment or yield of these plants, causing damage to other crops in agricultural areas or to native vegetation. They might also increase competition for desirable animals and reduce biodiversity.

128. Since severe infestations with arthropod canola pests and mouse plagues occur under current conditions, these harms are not expected to be greater as a consequence of the proposed field trial.

Conclusion

129. Risk scenario 2 is not identified as a substantive risk because of the limited ability of a few pest species to access GM canola seeds due to the proposed limits and controls, the proposed locations of the release, the lack of a sustained benefit for animals after intermittent consumption of small amounts of ω-3 LC-PUFAs and the importance of environmental conditions other than food quality on

pest populations. Therefore, this risk could not be greater than negligible and does not warrant further assessment.

Risk scenario 3

Risk source	GM canola expressing the introduced genes for an altered ω-3 seed oil profile
Causal pathway	⬇ Cultivation of GM canola at trial sites ⬇ Dispersal of GM seed outside trial limits ⬇ Establishment of populations of volunteer GM plants expressing the introduced genes in the environment ⬇
Potential harm	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable plants

Risk source

130. The source of potential harm for this postulated risk scenario is GM canola expressing the introduced genes for an altered ω-3 seed oil profile.

Causal pathway

131. GM canola modified for altered seed oil content would be cultivated at the trial sites and GM canola seeds could be dispersed outside the trial limits. If GM canola seeds were dispersed outside the trial sites or persisted at a site after completion of the trial, the seed could germinate. These plants could spread and persist and get established in the environment. People and other desirable organisms could be exposed to the introduced genes and end products outside trial limits.

Dispersal outside the trial site

132. Dispersal of viable GM canola seed outside the trial site could occur in a variety of ways, including movement of seeds by human activity, animal activity and endozoochory (dispersal through ingestion by animals), or spread of residual harvest seeds by high winds or flooding.

Potential dispersal by human activity

133. Human activity is considered the most significant method of long-distance seed dispersal for canola outside the trial limits (OGTR 2013b). It is possible for volunteer canola populations to establish due to seed spillage along the transport route and during the use of agricultural equipment (OGTR, 2017a). To reduce dispersal of GM plant material by humans, trial sites access will be only granted to trained and authorised personnel. Dispersal of GM plant material by authorised people entering the proposed trial site would be minimised by cleaning all equipment used, including clothing. All GM plant material would be transported in accordance with the Regulator's transport guidelines to reduce the opportunity for its dispersal.

Potential dispersal by animal activity or endozoochory

134. Canola seeds are not sticky, and lack burrs and hooks which can contribute to seed dispersal by attaching to animal fur or feathers (Howe & Smallwood 1982). These characteristics are not expected to be altered in the GMOs.

135. As discussed in the RARMP for DIR 123 (OGTR 2013b) and in Risk scenario 2 of this RARMP, animals such as kangaroos, feral pigs, emus or other birds may occasionally eat canola. 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 possible at very low levels (Twigg et al., 2008; Twigg et al., 2009). The viability of canola seed after passing through the digestive gut of animals is poorly

understood, but some studies support that seeds are unlikely to be viable after digestion. A study of several species of native doves, ducks and finches feed on canola found that only wood ducks (*Chenonetta jubata*) excreted intact seed, representing less than 0.01% of the seed ingested (Twigg et al., 2008). From those seeds, the germination potential was reduced to less than 50%. These results indicate that less than 0.005% viable canola is likely to be spread by the species studied.

Potential dispersal by flooding or high winds

136. Canola seeds also lack specialised structures that would assist their dispersal by wind. However, canola may be windrowed prior to harvesting, and under strong wind conditions plant material could disperse beyond trial boundaries. Establishment of monitoring zones around trial sites, which are inspected during and after trials, and post-harvest cleaning of all areas onto which GM canola seeds may have been dispersed would manage potential for dispersal of GM canola seeds.

137. It is also possible that heavy rains or flooding could transport GM canola seeds away from trial sites (OGTR, 2017a). Canola seedlings are sensitive to waterlogged soil, but if the flooding does not occur over an extended time period, the GM canola could survive. However, canola needs continued irrigation or rainfall to persist. The applicant has proposed to locate the trial sites at least 50 m from permanent natural waterways to minimise the potential for seed dispersal during flooding.

138. Non-GM canola is a poor competitor and feral populations rely on recurrent spillages to persist (Yoshimura et al., 2006). It is also not a significant weed, and it is not likely to become invasive (Busi and Powles, 2016). The genetic modifications are not expected to increase the potential of the GM canola to spread and persist outside cultivation.

Persistence at the trial sites

139. Persistence of GMOs at the trial sites after the field experiment is finished could occur if seeds in the seed bank were dormant. Canola generally does not exhibit primary dormancy, but secondary dormancy has been described (OGTR, 2017a). A study carried out in western Canada revealed that secondary seed dormancy prolonged persistence of volunteer canola plants (Gulden et al., 2003). Persisting canola seed banks have been shown to significantly contribute to the dynamics of feral canola populations (Pivard et al., 2008). A long-term monitoring study in Germany detected GM canola volunteers in arable fields for up to fifteen years after the field trial concluded, but did not detect spatial dispersion (Belter, 2016). In Australia, volunteers can be found for up to 3 years after growing canola due to persistence in seed banks, though the majority of volunteer seedlings emerge the year following a canola crop (AOF, 2014).

140. Seed traits such as oil composition could alter seed dormancy (Linder and Schmitt, 1995; Simard, 2010). This could have an impact on GM seedbank persistence, increasing the probability for weediness compared to non-GM canola and commercially approved GM canola. To minimise the likelihood of secondary seed dormancy and persistence of GM canola after completion of the trial, germination of any residual GM seed would be promoted by light post-harvest tillage and irrigation. Further management would involve post-harvest monitoring inspections of the trial sites and destruction of canola volunteers for at least 2 years, and until no volunteers are observed in the most recent 12 month period.

Potential harm

141. If the GM seeds germinated and gave rise to volunteers expressing the introduced genes, these could spread and establish in the environment. If GM volunteers spread and established in the environment, there could be adverse environmental impacts on native or other desirable vegetation due to weediness of the GM volunteers or due to increased populations of canola pests. People and other desirable organisms exposed to the introduced genes, proteins and end products may show increased toxic or allergic reactions compared to those exposed to non-GM canola or commercially approved GM canola.

Toxicity and allergenicity to humans and other desirable organisms

142. As discussed in Risk scenario 1, it is not expected that proteins encoded by the introduced genes, encoded proteins and their end products will have increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms.

Potential for reduced establishment or yield of desirable plants

143. Volunteer GM canola could spread and persist as a weed in nature reserves, displacing native vegetation. However, even if a spillage occurs, GM canola in Australia has low likelihood to become invasive, and volunteers can be effectively controlled by current weed management practices, including a mixture of herbicide modes of action (Busi and Powles, 2016). Alternatively, native and other desirable plants may be adversely affected by pests gaining an advantage over those eating non-GM canola or commercially approved GM canola, and an increase in their population beyond current conditions. The latter was discussed in Risk scenario 2 and concluded that intermittent exposure of pests to the GM canola seeds would not lead to sustained benefits to many pest species and would not alter their response to environmental conditions other than quality of food. Therefore, the risk of native and other desirable vegetation being adversely impacted by pests eating the GM canola seed would be no greater as a result of the proposed release.

144. It is possible for the GM traits to alter plant fitness and influence the reproductive success of the GM canola plants, thereby increasing its weediness potential (Busi and Powles, 2016; Sanyal and Decocq, 2016). It has been shown that drought stress is associated with lower content of oleic acid and content of higher polyunsaturated FA in canola seed oil (Aslam et al., 2009). It is then possible that higher PUFA content in the seed could lead to better drought tolerance. Higher content of linolenic acid and α-linolenic acid in seed has also been related to plant tolerance to cold and osmotic stress (Geilen et al., 2017). No information was provided by the applicant regarding the tolerance of the seeds to draught, cold or osmotic stresses. Therefore, this is an area of uncertainty for this risk assessment.

145. However, for an increase in weediness, these characteristics would need to be coupled with other mechanisms that increase the potential for spread and persistence in the environment, through changes in dispersal and establishment characteristics. These characteristics are not expected to change as a result of the introduced genes, either in individual lines or in a hybrid background. Furthermore, seed traits will not necessarily change the stress tolerance of the volunteer GM canola plants, which would have to overcome the same biotic and abiotic stresses that normally restrict their persistence (Chapter 1, Sections 6.1 and 6.2). For those reasons, GM canola would not be expected to lead to a reduction in the establishment or yield of desirable plants.

Conclusion

146. Risk scenario 3 is not identified as a substantive risk because the proposed limits and controls restrict the likelihood of dispersal and persistence of the GM canola after completion of the trial. Also, GM canola has limited ability to spread and persist outside cultivation, and it is susceptible to a variety of weed control measures. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Risk scenario 4

Risk source	GM canola expressing the introduced genes for an altered ω-3 seed oil profile
Causal pathway	⬇ Cultivation of GM canola at trial sites ⬇ GM canola pollen flow outside the trial site ⬇ Outcrossing with other sexually compatible plants ⬇ Establishment of populations of hybrid GM plants expressing the introduced genes in the environment

	⬇
Potential harm	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable plants

Risk source

147. The source of potential harm for this postulated risk scenario is GM canola expressing the introduced genes for an altered ω-3 seed oil profile.

Causal pathway

148. GM canola modified for altered seed oil content would be cultivated at the trial sites. Pollen from GM canola could be transferred outside the trial sites and fertilise sexually compatible plants, either non-GM canola, GM canola authorised for commercial release or plants from another sexually compatible species. Hybrid plants carrying the inserted genes could form the basis for the spread of these genes in other canola or other sexually compatible species and persist and get established in the environment. People and other desirable organisms could be exposed to the introduced genes and end products outside trial limits.

149. It should be noted that vertical gene flow *per se* is not considered an adverse outcome, but may be a link in a chain of events that may lead to an adverse outcome.

150. Although canola is predominantly self-pollinating, up to 30% of seeds can result from cross-pollination (OGTR, 2017a). Thus, gene flow via pollen is possible if pollen from GM plants fertilise other canola or sexually compatible plants or crops. Pollen can be transported by physical contact, wind or insect pollinators. Outcrossing occurs at low levels and decreases rapidly with distance, with the majority of cross-pollination occurring in less than 10 m (OGTR, 2017a). It is not expected that the introduced genes would alter the pollen dispersal characteristics of the GM canola.

151. As stated in Chapter 1, Section 6.4, if there is synchronicity of flowering, canola can hybridise under natural conditions with sexually compatible species, including commercial plantings of GM and non-GM canola (OGTR, 2017a). The frequency of hybridisation between GM canola and other Brassica species is expected to occur at low or very low levels. GM *B. napus x B. rapa* hybrids have been found in agricultural land and along roads in Canada, confirming the possibility of hybridisation between these two Brassica species (Yoshimura et al., 2006). However, the majority of small-seeded hybrids, such as self-pollinated hybrids or hybrids resulting from *B. napus* x *B. juncea* hybrids backcrossed with *B. juncea, are highly unlikely to* establish in nature (Wei and Darmency, 2008). Hybrids between *B. napus and Raphanus raphanistrum* (wild radish) are highly unlikely to occur in Australia (OGTR, 2017a). In field experiments in Australia, two *B. napus x R. raphanistrum* hybrids were detected from a total seed sample size of approximately 52 million (Rieger et al., 2001). Hybrids between *B. napus* and *B. juncea* (Liu et al., 2010) and between *B. napus* and *B. oleracea* have been detected (Ford et al., 2006).

152. The applicant has proposed a number of control measures that would restrict the potential for pollen flow and gene transfer to sexually compatible plants (Chapter 1, Section 3) as well as the persistence of hybrids. These include options of covering the flowering GM canola plants with insect-proof tents during flowering to prevent access by pollinators or planting a pollen trap of non-GM canola in combination with surrounding each trial site with a monitoring zone and an isolation zone within which canola crops will be grown. These measures will reduce the likelihood of hybridisation occurring between the GM canola and compatible species further. Control measures such as treating pollen trap plants as if they were the GMO would reduce the likelihood of any hybrids persisting.

Potential harm

153. Any GM canola hybrids may contain the introduced genes that could result in the expression of the encoded proteins and end products. If the introduced proteins led to increased weediness in the

hybrids, they could spread and harm native or other desirable vegetation. People and other desirable organisms may be exposed to these GM hybrids and the introduced proteins and end products proteins that may be toxic or allergenic to humans or toxic to other desirable organisms.

Toxicity and allergenicity to humans and other desirable organisms

154. Risk Scenario 1 did not identify toxicity or allergenicity of any of the introduced genes as a substantive risk. There is no evidence to suggest that combinations of these genes would result in the production of novel proteins, or that their expression would be altered in a hybrid background. The genes are sourced from common organisms present in the environment, suggesting that humans and other desirable organisms have a history of exposure to the introduced genes, encoded proteins and their end products.

Potential for reduced establishment or yield of desirable plants

155. As discussed in Risk scenario 3, it is highly unlikely that the altered seed oil profile will provide any significant advantage over non-GM seeds for germination and survival of the GM canola seeds. Similarly, the altered oil profile is unlikely to provide an advantage to GM canola hybrids. Hybrids expressing the introduced proteins are unlikely to show greater spread and persistence in nature reserves or to survive standard weed management practices for *Brassica* volunteers in agricultural settings. Similarly, as discussed in Risk scenario 2, pests may gain an advantage by eating GM canola hybrid seeds over those eating non-GM canola or commercially approved GM canola, and an increase in their population beyond current conditions may ensue. The latter was discussed in Risk scenario 2 and concluded that intermittent exposure of pests to the GM canola seeds would not lead to sustained benefits to many pest species and would not alter their response to environmental conditions other than quality of food. This would be similar for pests eating GM hybrid canola, and, therefore, the risk of native and other desirable vegetation being adversely impacted by pests eating the GM hybrid canola seed would be no greater as a result of the proposed release.

Conclusion

156. Risk scenario 4 is not identified as a substantive risk due to the proposed limits and controls designed to restrict pollen flow as well as the limited capacity of canola to outcross. GM hybrids are not likely to differ from the GM canola, for which Risk scenarios 1 and 3 did not identify toxicity, allergenicity or weediness as substantive risks. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

157. Uncertainty is an intrinsic property of risk analysis and is present in all aspects of risk analysis[3].

158. There are several types of uncertainty in risk analysis (Clark and Brinkley, 2001; Hayes, 2004; Bammer and Smithson, 2008). 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

[3] 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.

– perception – processing and interpreting risk is shaped by our mental processes and social/cultural circumstances, which vary between individuals and over time.

159. Uncertainty is addressed by approaches such as 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.

160. As field trials of GMOs are designed to gather data, there are generally data gaps when assessing the risks of a field trial application. However, field trial applications are required to be limited and controlled. Even if there is uncertainty about the characteristics of a GMO, limits and controls restrict exposure to the GMO, and thus decrease the likelihood of harm.

161. For DIR 163, uncertainty is noted particularly in relation to:

- potential for increased toxicity and allergenicity of the GM canola
- potential for increased weediness of GM canola
- potential for increased fitness of pests of GM canola.

162. Additional data, including information to address these uncertainties, may be required to assess possible future applications with reduced limits and controls, such as a larger scale trial or the commercial release of these GMOs.

163. Chapter 3, Section 4, discusses information that may be required for future release.

Section 4 Risk evaluation

164. 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.

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

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

166. Four risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the limits and controls proposed by the applicant, and considering both the short and long term, none of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 3, and include:

- none of the GM plant material would enter commercial human food or animal feed
- the proteins encoded by the introduced genes and the end products are not known to be toxic or allergenic
- the GM canola plants have limited ability to establish populations outside cultivation
- limits on the size, locations and duration of the release would be imposed
- the suitability of controls proposed by the applicant to restrict the spread and persistence of the GM canola and its genetic material will be assessed and, if necessary amended and
- no adverse health effects on people handling the GM plants in previous field trials of GM canola with altered oil content have been reported.

167. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM canola plants into the environment are considered to be negligible. The *Risk*

Analysis Framework (OGTR, 2013a) 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 additional 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[4].

[4] As none of the proposed dealings are considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator allowed 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities, and the public.

Chapter 3 Risk management plan

Section 1 Background

168. 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 licence conditions.

169. 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.

170. 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 are also required to be reported to the Regulator.

171. 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. 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

172. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people or the environment from the proposed field trial of GM canola. These risk scenarios were considered in the context of the scale of the proposed release (Chapter 1, Section 3.1), the proposed control measures (Chapter 1, Section 3.2), and the receiving environment (Chapter 1, Section 6), and considering both the short and the long term. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

Section 3 General risk management

173. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and the environment are negligible. Therefore, to maintain the risk context, licence conditions have been imposed to limit the release to the proposed size, location and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment. The conditions are discussed and summarised in this Chapter and listed in full in the licence.

3.1 Draft licence conditions to limit and control the release

174. Sections 3.1 and 3.2 of Chapter 1 provide details of the limits and controls proposed by Nuseed in the application. These are taken into account in the four risk scenarios postulated for the proposed release in Chapter 2. Many of these are discussed in the four risk scenarios considered for the proposed release in Chapter 2.

175. The following proposed limits and controls were considered in the risk management plan for DIR 123, and imposed as licence conditions in licence DIR 123. This release did not result in any

reported adverse effects. It is considered that these measures would also be effective in limiting and controlling the GM canola proposed for release here.

General conditions

- Permitting only trained and authorised personnel to conduct activities with a GMO (DIR 123 licence Conditions 6, 12, 14, 15, 16 and 20).

176. This addresses potential exposure of humans to the GMOs (Risk scenario 1) and accidental dispersal of the GMOs (Risk scenario 3).

Limits

- Not using the GM canola and any products from it in commercial food or feed (DIR 123 licence conditions 24 and 26),
- conducting food or feed testing once the appropriate authorisation has been obtained (DIR 123 licence condition 25),
- limiting exposure of people and animals to 5 years (DIR 123 licence condition 23),
- limiting the trial sites to ten sites of 10 ha and ten sites of 5 ha per year[5], and
- limiting the release to up to 20 locations per year, selected from 95 LGAs in NSW, VIC and QLD[6]. The QLD locations differ from locations in DIR 123. However, these locations are in Australian canola growing areas and therefore considered appropriate.

177. The limited size and duration of the trial would minimise the potential exposure of humans and other desirable organisms as well as pests to the GMOs (Risk scenarios 1 and 2), in addition to the potential for dispersal, persistence and outcrossing of the GMOs (Risk scenarios 3 and 4). Limits on food and feed would minimise exposure of desirable animals to the GM canola by consumption (Risk scenario 1).

Control measures regarding pollen and seed dispersal during cultivation

- Locating the trial sites at least 50 m away from waterways (DIR 123 licence condition 28),
- restricting pollen flow by a combination of measures (see DIR 123 licence conditions 2, 29, 30 and Figure 1).
- treating non-GM canola and pollen trap plants, if used, like the GM canola (DIR 123 licence conditions 31 and 35),
- harvesting the GM canola separately from other canola crops (DIR 123 licence condition 36),
- minimising wind or water dispersal of windrowed material (DIR 123 licence condition 37),
- notifying the Regulator of the intended method of harvest (DIR 123 licence condition 54),
- restricting grazing of livestock (DIR 123 licence conditions 49 and 50),
- destroying the GM canola not required for further evaluation or future trials using previously approved methods (DIR 123 licence conditions 41 and 42, and the definition of “destroy” under condition 2),
- cleaning trial sites following harvest, and cleaning equipment used in connection with the GMOs as soon as practicable and before use by any other purpose (DIR 123 licence conditions 40 and 40A).

178. The definition of pollen trap plants has been expanded to include both non-GM canola and GM canola approved for commercial release by the Regulator. These pollen trap plants are subject to the same destruction and post-cleaning conditions as the GMO in the planting area.

179. For destruction of the GM canola seeds by burial, wetting of the seeds at time of burial has been imposed. This wetting will encourage decomposition of the seeds in the burial pit to reduce

[5, 6] These limits differ from the previously approved licence DIR 123.

persistence. These proposed controls would minimise persistence or dispersal of GM canola seed (Risk scenario 3) and pollen-mediated gene flow to other canola crops and sexually compatible species (Risk scenario 4).

Control measures regarding the dispersal of the GMOs during transport or storage

- Transporting and storing the GM canola in accordance with the current Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs (2011)* (DIR 123 licence Conditions 45 and 46).

180. These are requirements imposed in many field trials of GM crops which minimise the potential for exposure of people and other organisms to the GMOs (Risk scenarios 1 and 2), dispersal into the environment (Risk scenario 3), and gene transfer (Risk scenario 4).

Control measures regarding persistence of the GMOs or GM volunteers post-cleaning and use of areas post-harvest

- Post-harvest surface tilling of the planting area and pollen trap, if used, and other areas where the GMO was dispersed to encourage seed germination (DIR 123 licence condition 48),
- post-harvest monitoring monthly for at least 2 years until the site is signed off by the Regulator, after no volunteers are observed in the most recent 12 month period (DIR 123 licence conditions 48 and 53), and
- planting only crops permitted by the Regulator's Policy on Post- Harvest Crops (2013) permitted on GM Brassica trial sites during the post-harvest monitoring period (DIR 123 licence condition 48(e)).

181. These control measures would minimise the dispersal and persistence of the GM canola in the environment (Risk scenarios 3 and 4). Enclosing trial sites with a fence and gates, and not permitting grazing in the planting area or pollen trap at any time prior to the Regulator issuing a sign-off for these areas would minimise the exposure of livestock and wild animals to the GMOs by consumption (Risk scenario 1) as well as the potential for dispersal of GM seeds (Risk scenario 3).

3.2 Other risk management considerations

182. 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
- contingency plans
- identification of the persons or classes of persons covered by the licence
- reporting requirements
- access for the purpose of monitoring for compliance.

3.2.1 Applicant suitability

183. 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, for either an individual applicant or a body corporate, include:

- any relevant convictions of the applicant
- 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.

184. The conditions of the licence include a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

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

3.2.2 Contingency plan

186. Nuseed is required to submit a contingency plan to the Regulator before planting the GMOs. This plan would detail measures to be undertaken in the event of any unintended presence of the GM canola outside permitted areas.

187. Nuseed is also required to provide the Regulator with a method to reliably detect the GMOs or the presence of the genetic modifications in a recipient organism. This methodology is required before planting the GMOs.

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

188. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to growing the GMOs, Nuseed would be required to provide a list of people and organisations that will be covered by the licence, or the function or position where names are not known at the time.

3.2.4 Reporting requirements

189. 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 trial
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the trial.

190. A number of written notices are also required under the licence to assist the Regulator in designing and implementing a monitoring program for all licensed dealings. The notices include:

- expected and actual dates of planting
- details of areas planted to the GMOs
- expected dates of flowering
- expected and actual dates of harvest, method of harvest and cleaning after harvest
- details of inspection activities.

3.2.5 Monitoring for compliance

191. 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 inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing. Post-release monitoring continues until the Regulator is satisfied that all the GMOs resulting from the authorised dealings have been removed from the release sites.

192. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

193. 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 health and safety of people or the environment could result.

Section 4 Issues to be addressed for future releases

194. Additional information has been identified that may be required to assess an application for a commercial release of these GM canola lines, or to justify a reduction in limits and controls. This includes:

- additional molecular and biochemical characterisation of the GM canola plants, particularly with respect to potential for increased toxicity and allergenicity and
- additional phenotypic characterisation of the GM canola plants, particularly with respect to traits that may contribute to seed persistence, weediness and pests fitness.

Section 5 Conclusions of the consultation RARMP

195. The RARMP concludes that this limited and controlled 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.

196. Conditions have been imposed to limit the release to the proposed size, location and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment, as these were important considerations in establishing the context for assessing the risks.

References

ABARES (2018). Australian Crop Report No.185, February 2018. Report No. 182. (Canberra, Australia: Department of Agriculture and Water Resources).

Alizadeh, A., Esmaeili, V., Shahverdi, A., and Rashidi, L. (2014). Dietary fish oil can change sperm parameters and fatty acid profiles of ram sperm during oil consumption period and after removal of oil source. Cell journal *16*, 289-298.

Alizadeh, A., Taleb, Z., Ebrahimi, B., Esmaeili, V., Shaverdi, A., Nasr, J., Kheimeh, A*., et al.* (2016). Dietary vitamin E is more effective than Omega-3 and Omega-6 Fatty Acid for improving the kinematic characteristics of rat sperm. Cell journal *18*, 262-270.

AOF (2014). Canola volunteer control. (New South Wales, Australia: Australian Oilseeds Federation).

AOF (2017). Crops Report. (New South Wales, Australia: Australian oilseeds Federation).

Arts, J.H.E., Mommers, C., and de Heer, C. (2006). Dose-response relationships and threshold levels in skin and respiratory allergy. Critical Reviews in Toxicology *36*, 219-251.

Aslam, M.N., Nelson, M.N., Kailis, S.G., Bayliss, K.L., Speijers, J., and Cowling, W.A. (2009). Canola oil increases in polyunsaturated fatty acids and decreases in oleic acid in drought-stressed Mediterranean-type environments. Plant Breeding *128*, 348-355.

Bammer, G., and Smithson, M. (2008). Uncertainty and risk: Multidisciplinary perspectives (London: Earthscan).

Belter, A. (2016). Long-term monitoring of field trial sites with genetically modified oilseed rape (*Brassica napus* L.) in saxony-Anhalt, Germany. Fifteen years persistence to date but no spatial dispersion. Genes *7*, 1-13.

Berkley, S.F., Hightower, A.W., Beier, R.C., Fleming, D.W., Brokopp, C.D., Ivie, G.W., and Broome, C.V. (1986). Dermatitis in grocery workers associated with high natural concentrations of furanocoumarins in celery. Annals of internal medicine *105*, 351-355.

Bevan, M. (1984). Binary *Agrobacterium* vectors for plant transformation. Nucleic Acids Research *12*, 8711-8721.

Blesbois, E., Douard, V., Germain, M., Boniface, P., and Pellet, F. (2004). Effects of n-3 polyunsaturated dietary supplementation on the reproductive capacity of male turkeys. Theriogenology *61*, 537-549.

Bomford, M., and Sinclair, R. (2002). Australian research on bird pests: impact, management and future directions. Emu *102*, 29-45.

Breyer, D., Kopertekh, L., and Reheul, D. (2014). Alternatives to antibiotic resistance marker genes for *in vitro* selection of genetically modified plants – Scientific developments, current use, operational access and biosafety considerations. Critical Reviews in Plant Sciences *33*, 286-330.

Busi, R., and Powles, S.B. (2016). Transgenic glyphosate-resistant canola (*Brassica napus*) can persist outside agricultural fields in Australia. Agriculture, Ecosystems & Environment *220*, 28-34.

Byelashov, O.A., Sinclair, A.J., and Kaur, G. (2015). Dietary sources, current intakes, and nutritional role of omega-3 docosapentaenoic acid. Lipid Technology *27*, 79-82.

Byrne, C.J., Fair, S., English, A.M., Holden, S.A., Dick, J.R., Lonergan, P., and Kenny, D.A. (2017). Dietary polyunsaturated fatty acid supplementation of young post-pubertal dairy bulls alters the fatty acid composition of seminal plasma and spermatozoa but has no effect on semen volume or sperm quality. Theriogenology *90*, 289-300.

Cai, L., Zhou, B., Guo, X., Dong, C., Hu, X., Hou, M., and Liu, S. (2008). Pollen-mediated gene flow in Chinese commercial fields of glufosinate-resistant canola (*Brassica napus*). Chinese Science Bulletin *53*, 2333.

Cardoso, T.F., Varela, A.S., Jr., Silva, E.F., Vilela, J., Hartmann, A., Jardim, R.D., Colares, E.P.*, et al.* (2014). Influence of mineral, olive or sunflower oils on male reproductive parameters in vitro-the wild rodent *Calomys laucha*. Andrologia *46*, 722-725.

Carragher, J.F., Muhlhausler, B.S., Geier, M.S., House, J., Hughes, R.J., and Gibson, R.A. (2015). Effect of dietary ALA on growth rate, feed conversion ratio, mortality rate and breast meat omega-3 LCPUFA content in broiler chickens, Vol 56.

Carrie, I., Smirnova, M., Clement, M., De, J.D., Frances, H., and Bourre, J.M. (2002). Docosahexaenoic acid-rich phospholipid supplementation: effect on behavior, learning ability, and retinal function in control and n-3 polyunsaturated fatty acid deficient old mice. Nutritional neuroscience *5*, 43-52.

Castellano, C.A., Audet, I., Bailey, J.L., Chouinard, P.Y., Laforest, J.P., and Matte, J.J. (2010). Effect of dietary n-3 fatty acids (fish oils) on boar reproduction and semen quality. Journal of animal science *88*, 2346-2355.

CERA (2011). A Review of the Environmental Safety of the PAT Protein. (Center for Environmental Risk Assessment, ILSI Research Foundation).

Cerolini, S., Pizzi, F., Gliozzi, T., Maldjian, A., Zaniboni, L., and Parodi, L. (2003). Lipid manipulation of chicken semen by dietary means and its relation to fertility: a review. World's Poultry Science Journal *59*, 65-75.

Chaudhary, S., Van Rooijen, G., Moloney, M., and Singh, S. (2001). Flax seed specific promoters. Patent No. WO 2001/016340 (International Patent Application).

Cho, M.J., Widholm, J.M., and Vodkin, L.O. (1995). Cassettes for seed-specific expression tested in transformed embryogenic cultures of soybean. Plant Molecular Biology Reporter *13*, 255-269.

Clark, A.J., and Brinkley, T. (2001). Risk management: for climate, agriculture and policy. (Canberra: Commonwealth of Australia).

Colombo, S.M., Campbell, L.G., Murphy, E.J., Martin, S.L., and Arts, M.T. (2018). Potential for novel production of omega-3 long-chain fatty acids by genetically engineered oilseed plants to alter terrestrial ecosystem dynamics. Agricultural Systems *164*, 31-37.

Coutu, C., Brandle, J., Brown, D., Brown, K., Miki, B., Simmonds, J., and Hegedus, D.D. (2007). pORE: a modular binary vector series suited for both monocot and dicot plant transformation. Transgenic research *16*, 771-781.

Cutuli, D., De Bartolo, P., Caporali, P., Laricchiuta, D., Foti, F., Ronci, M., Rossi, C.*, et al.* (2014). n-3 polyunsaturated fatty acids supplementation enhances hippocampal functionality in aged mice. Front Aging Neurosci *6*, 220.

DeMar, J.C., Jr., DiMartino, C., Baca, A.W., Lefkowitz, W., and Salem, N., Jr. (2008). Effect of dietary docosahexaenoic acid on biosynthesis of docosahexaenoic acid from alpha-linolenic acid in young rats. J Lipid Res *49*, 1963-1980.

Dyall, S.C. (2015). Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA. Frontiers in Aging Neuroscience *7*, 1-15.

EFSA (2009). Scientific opinion of the GMO and BIOHAZ Panels on the "Use of antibiotic resistance genes as marker genes in genetically modified plants". European Food Safety Authority *1034*, 1-82.

Fair, S., Doyle, D.N., Diskin, M.G., Hennessy, A.A., and Kenny, D.A. (2014). The effect of dietary n-3 polyunsaturated fatty acids supplementation of rams on semen quality and subsequent quality of liquid stored semen. Theriogenology *81*, 210-219.

Felsot, A.S. (2000). Insecticidal genes part 2: Human health hoopla. Agrichemical & Environmental News *168*, 1-7.

Fiocchi, A., Dahdah, L., Riccardi, C., Mazzina, O., and Fierro, V. (2016). Preacutionary labelling of cross-reactive foods: The case of rapeseed. Asthma research and practice *2*, 13.

Ford, C.S., Allainguillaume, J., Grilli-Chantler, P., Cuccato, G., Allender, C.J., and Wilkinson, M.J. (2006). Spontaneous gene flow from rapeseed (*Brassica napus*) to wild *Brassica oleracea*. Proceedings of the Royal Society B: Biological Sciences *273*, 3111-3115.

FSANZ (2017). Approval report – Application A1143: Food derived from DHA Canola Line NS-B50027-4 (Canberra, Australia: Food Standards Australia New Zealand).

Funk, T., Wenzel, G., and Schwarz, G. (2006). Outcrossing frequencies and distribution of transgenic oilseed rape (*Brassica napus* L.) in the nearest neighbourhood. European Journal of Agronomy *24*, 26-34.

Gallie, D.R., Sleat, D.E., Watts, J.W., Turner, P.C., and Wilson, T.M. (1987). A comparison of eukaryotic viral 5'-leader sequences as enhancers of mRNA expression in vivo. Nucleic Acids Res *15*, 8693-8711.

Geilen, K., Heilmann, M., Hillmer, S., and Böhmer, M. (2017). WRKY43 regulates polyunsaturated fatty acid content and seed germination under unfavourable growth conditions. Scientific Reports *7*, 14235.

Gliozzi, T.M., Zaniboni, L., Maldjian, A., Luzi, F., Maertens, L., and Cerolini, S. (2009). Quality and lipid composition of spermatozoa in rabbits fed DHA and vitamin E rich diets. Theriogenology *71*, 910-919.

GRDC (2009). Canola best practice management guide for south-eastern Australia. (Canberra, Australia: Grains Research & Development Corporation).

GRDC (2011). Minimising crop damage by mice. (Canberra, Australia: Grains Research & Development Corporation).

GRDC (2017). GRDC Northern Canola GrowNotes. (Canberra, Australia: Grains Research & Development Corporation).

Gulden, R.H., Shirtliffe, S.J., and Thomas, A.G. (2003). Secondary seed dormancy prolongs persistence of volunteer canola in western Canada. Weed Science *51*, 904-913.

Gylling, H. (2006). Rapeseed oil does not cause allergic reactions. Allergy *61*, 895.

Hall, G.J., Allen, G.C., Loer, D.S., Thompson, W.F., and Spiker, S. (1991). Nuclear scaffolds and scaffold-attachment regions in higher plants. Proc Natl Acad Sci U S A *88*, 9320-9324.

Halweg, C., Thompson, W.F., and Spiker, S. (2005). The rb7 matrix attachment region increases the likelihood and magnitude of transgene expression in tobacco cells: a flow cytometric study. Plant Cell *17*, 418-429.

Hayes, K.R. (2004). Ecological implications of GMOs: robust methodologies for ecological risk assessment. Best practice and current practice in ecological risk assessment for genetically modified organisms. (Tasmania: CSIRO Division of Marine Research).

Hixson, S.M., Shukla, K., Campbell, L.G., Hallett, R.H., Smith, S.M., Packer, L., and Arts, M.T. (2016). Long-Chain Omega-3 polyunsaturated fatty acids have developmental effects on the crop pest, the cabbage white butterfly *Pieris rapae*. PLoS One *11*, e0152264.

Howlett, B., Ballinger, D., and Barbetti, M. (1999). Diseases of Canola. In Canola in Australia: The first thirty years, P.A. Salisbury, T.D. Potter, G. McDonald, and A.G. Green, eds. (Australian Oilseeds Federation), pp. 47-52.

Huang, C.W., Chien, Y.S., Chen, Y.J., Ajuwon, K.M., Mersmann, H.M., and Ding, S.T. (2016). Role of n-3 polyunsaturated fatty acids in ameliorating the obesity-induced metabolic syndrome in animal models and humans. International journal of molecular sciences *17*.

Hughbanks-Wheaton, D.K., Birch, D.G., Fish, G.E., Spencer, R., Pearson, N.S., Takacs, A., and Hoffman, D.R. (2014). Safety assessment of docosahexaenoic acid in X-linked retinitis pigmentosa: the 4-year DHAX trial. Investigative ophthalmology & visual science *55*, 4958-4966.

Hüsken, A., and Dietz-Pfeilstetter, A. (2007). Pollen-mediated intraspecific gene flow from herbicide resistant oilseed rape (*Brassica napus* L.). Transgenic Research *16*, 557-569.

Jackson, C. (2009). Assessing and quantifying canola crop damage by Carnaby's cockatoo *Calyptorhynchus latirostris* in the south-west of Western Australia. Masters in Applied Science, University of Sydney.

Kay, R., Chan, A., Daly, M., and McPherson, J. (1987). Duplication of CaMV 35S promoter sequences creates a strong enhancer for plant genes. Science *236*, 1299-1302.

Keese, P.K., Robold, A.V., Myers, R.C., Weisman, S., and Smith, J. (2014). Applying a weed risk assessment approach to GM crops. Transgenic Research *23*, 957-969.

Khoshvaght, A., Towhidi, A., Zare-shahneh, A., Noruozi, M., Zhandi, M., Dadashpour Davachi, N., and Karimi, R. (2016). Dietary n-3 PUFAs improve fresh and post-thaw semen quality in Holstein bulls via alteration of sperm fatty acid composition. Theriogenology *85*, 807-812.

Kim, S., Yamaoka, Y., Ono, H., Kim, H., Shim, D., Maeshima, M., Martinoia, E.*, et al.* (2013). AtABCA9 transporter supplies fatty acids for lipid synthesis to the endoplasmic reticulum. Proceedings of the National Academy of Sciences of the United States of America *110*, 773-778.

Latitude_42 (2011). Pest Risk Assessment: Crested Pigeon (*Ocyphaps lophotes*). Latitude 42 Environmental Consultants Pty Ltd (Hobart, Tasmania).

Lin, Y., Cheng, X., Mao, J., Wu, D., Ren, B., Xu, S.-Y., Fang, Z.-F.*, et al.* (2016). Effects of different dietary n-6/n-3 polyunsaturated fatty acid ratios on boar reproduction. Lipids in Health and Disease *15*, 31.

Linder, C.R., and Schmitt, J. (1995). Potential persistence of escaped transgenes: performance of transgenic oil-modified *Brassica* seeds and seedlings. Ecological Applications *5*, 1056-1068.

Liu, Y.B., Wei, W., Ma, K.P., and Darmency, H. (2010). Backcrosses to *Brassica napus* of hybrids between *B. juncea* and *B. napus* as a source of herbicide-resistant volunteer-like feral populations. Plant Science *179*, 459-465.

Martinez Del Rio, C., and McWilliams, S.R. (2016). How essential fats affect bird performance and link aquatic ecosystems and terrestrial consumers. Proc Natl Acad Sci U S A *113*, 11988-11990.

McCann, J.C., and Ames, B.N. (2005). Is docosahexaenoic acid, an n−3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. The American Journal of Clinical Nutrition *82*, 281-295.

Meesapyodsuk, D., and Qiu, X. (2016). Biosynthetic mechanism of very long chain polyunsaturated fatty acids in Thraustochytrium sp. 26185. Journal of Lipid Research *57*, 1854-1864.

Miles, M., and McDonald, G. (1999). Insect Pests. In Canola in Australia: The First Thirty Years, P.A. Salisbury, T.D. Potter, G. McDonald, and A.G. Green, eds., pp. 53-58.

OECD (1999). Consensus document on general information concerning the genes and their enzymes that confer tolerance to phosphinothricin herbicide. Report No. ENV/JM/MONO(99)13. (Organisation for Economic Cooperation and Development).

OECD (2011). Revised consensus document on compositional considerations for new varieties of low erucic acid rapeseed (canola): Key food and feed nutrients, anti-nutrients and toxicants. Report No. ENV/JM/MONO(2011)55. (Organisation for Economic Cooperation and Development).

OGTR (2003). Risk Assessment and Risk Management Plan for DIR 021/2002: Commercial release of InVigor® hybrid canola (*Brassica napus* L.) for use in the Australian cropping system. (Canberra, Australia: Office of the Gene Technology Regulator).

OGTR (2011). Risk Assessment and Risk Management Plan for DIR 108: Commercial release of canola genetically modified for herbicide tolerance and a hybrid breeding system (InVigor® x Roundup Ready® canola). (Canberra, Australia: Office of Gene Technology Regulator).

OGTR (2013a). Risk Analysis Framework 2013, 4th edn (Canberra: Office of the Gene Technology Regulator).

OGTR (2013b). Risk Assessment and Risk Management Plan for DIR 123: Limited and controlled release of canola genetically modified for altered oil content. (Canberra, Australia: Office of the Gene Technology Regulator).

OGTR (2016). Risk Assessment and Risk Management Plan for DIR 138: Commercial release of canola genetically modified for dual herbicide tolerance and a hybrid breeding system (InVigor® x TruFlex™ Roundup Ready®). (Canberra, Australia: Office of the Gene Technology Regulator).

OGTR (2017a). The Biology of *Brassica napus* L. (canola) and *Brassica juncea* (L.) Czern. & Coss. (Indian mustard). (Canberra, Australia: Office of the Gene Technology Regulator).

OGTR (2017b). Fact sheet - GM canola approved for commercial release in Australia (Canberra, Australia: Office of the Gene Technology Regulator).

OGTR (2017c). Risk Assessment and Risk Management Plan for DIR 153: Limited and controlled release of sorghum genetically modified for grain quality traits. (Canberra, Australia: Office of the Gene Technology Regulator).

OGTR (2017d). Risk Assessment Reference: Marker Genes in GM Plants. (Canberra, Australia: Office of the Gene Technology Regulator).

OGTR (2018a). 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). (Canberra, Australia: Office of the Gene Technology Regulator).

OGTR (2018b). Risk Assessment Reference: Methods of Plant Genetic Modification. (Canberra, Australia: Office of the Gene Technology Regulator).

Olubowale, O.S., Fh, d., Greyling, J., Hugo, A., Jooste, A.M., and Raito, M. (2014). The effect of dietary lipid sources on layer fertility and hatchability, Vol 44.

Petrie, J.R., Liu, Q., Mackenzie, A.M., Shrestha, P., Mansour, M.P., Robert, S.S., Frampton, D.F*., et al.* (2010a). Isolation and characterisation of a high-efficiency desaturase and elongases from microalgae for transgenic LC-PUFA production. Mar Biotechnol (NY) *12*, 430-438.

Petrie, J.R., Shrestha, P., Mansour, M.P., Nichols, P.D., Liu, Q., and Singh, S.P. (2010b). Metabolic engineering of omega-3 long-chain polyunsaturated fatty acids in plants using an acyl-CoA Delta 6-desaturase with omega 3-preference from the marine microalga *Micromonas pusilla*. Metab Eng *12*, 233-240.

Petrie, J.R., Shrestha, P., Zhou, X.R., Mansour, M.P., Liu, Q., Belide, S., Nichols, P*., et al.* (2012). Metabolic engineering plant seeds with fish oil-like levels of DHA. PLoS ONE *7*, e49165. doi:49110.41371/journal.pone.0049165.

Petrie, J.R., and Singh, S.P. (2011). Expanding the docosahexaenoic acid food web for sustainable production: engineering lower plant pathways into higher plants. AoB Plants *2011*, 1-11.

Pivard, S., Adamczyk, K., Lecomte, J., Lavigne, C., Bouvier, A., Deville, A., Gouyon, P.H*., et al.* (2008). Where do the feral oilseed rape populations come from? A large-scale study of their possible origin in a farmland area. Journal of Applied Ecology *45*, 476-485.

Rieger, M.A., Lamond, M., Preston, C., Powles, S.B., and Roush, R. (2002). Pollen-mediated movement of herbicide resistance between commercial canola fields. Science *296*, 2386-2388.

Rieger, M.A., Potter, T.D., Preston, C., and Powles, S.B. (2001). Hybridisation between *Brassica napus* L. and *Raphanus raphanistrum* L. under agronomic field conditions. Theoretical and Applied Genetics *103*, 555-560.

Rodemer, C., Thai, T.P., Brugger, B., Kaercher, T., Werner, H., Nave, K.A., Wieland, F*., et al.* (2003). Inactivation of ether lipid biosynthesis causes male infertility, defects in eye development and optic nerve hypoplasia in mice. Human molecular genetics *12*, 1881-1895.

Rogers, S.G., O'Connell, K., Horsch, R.B., and Fraley, R.T. (1985). Investigation of factors involved in foreign protein expression in transformed plants. In Biotechnology in Plant Science, P. Day, and A. Hollaender, eds. (Academic Press), pp. 219-226.

Rossak, M., Smith, M., and Kunst, L. (2001). Expression of the FAE1 gene and FAE1 promoter activity in developing seeds of *Arabidopsis thaliana*. Plant Mol Biol *46*, 717-725.

Ruiz-Lopez, N., Haslam, R.P., Usher, S.L., Napier, J.A., and Sayanova, O. (2013). Reconstitution of EPA and DHA biosynthesis in arabidopsis: iterative metabolic engineering for the synthesis of n-3 LC-PUFAs in transgenic plants. Metab Eng *17*, 30-41.

Ruiz-Lopez, N., Usher, S., Sayanova, O.V., Napier, J.A., and Haslam, R.P. (2015). Modifying the lipid content and composition of plant seeds: engineering the production of LC-PUFA. Applied Microbiology and Biotechnology *99*, 143-154.

Sanders, P.R., Winter, J.A., Barnason, A.R., Rogers, S.G., and Fraley, R.T. (1987). Comparison of cauliflower mosaic virus 35S and nopaline synthase promoters in transgenic plants. Nucleic Acids Research *15*, 1543-1558.

Sanyal, A., and Decocq, G. (2016). Adaptive evolution of seed oil content in angiosperms: accounting for the global patterns of seed oils. BMC Evol Biol *16*, 187.

Sayanova, O.V., and Napier, J.A. (2004). Eicosapentaenoic acid: biosynthetic routes and the potential for synthesis in transgenic plants. Phytochemistry *65*, 147-158.

Schnell, J., Steele, M., Bean, J., Neuspiel, M., Girard, C., Dormann, N., Pearson, C*., et al.* (2015). A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments. Transgenic Research *24*, 1-17.

Seligman, P.J., Mathias, C.G.T., O'Malley, M.A., Beier, R.C., Fehrs, L.J., Serrill, W.S., and Halperin, W.E. (1987). Phytophotodermatitis from celery among grocery store workers. Archives of dermatology *123*, 1478-1482.

Shahidi, F., and Ambigaipalan, P. (2018). Omega-3 Polyunsaturated fatty acids and their health benefits. Annual review of food science and technology *9*, 345-381.

Sharma, R. (2013). Omega-3 Fatty Acids in prevention of cardiovascular disease in humans: chemistry, dyslipidemia A2 In Bioactive Food as Dietary Interventions for Cardiovascular Disease, V.R. Preedy, ed. (San Diego: Academic Press), pp. 465-489.

Simard, M.-J. (2010). Gene flow between crops and their wild relatives. Evolutionary Applications *3*, 402-403.

Society of Toxicology (2003). Society of Toxicology position paper: The safety of genetically modified foods produced through biotechnology. Toxicological Sciences *71*, 2-8.

Stalberg, K., Ellerstrom, M., Josefsson, L.G., and Rask, L. (1993). Deletion analysis of a 2S seed storage protein promoter of *Brassica napus* in transgenic tobacco. Plant Mol Biol *23*, 671-683.

Standards Australia, Standards New Zealand, and CRC for Australian Weed Management (2006). HB 294:2006 National Post-Border Weed Risk Management Protocol (Standards Australia and Standards New Zealand).

Steiner, H.Y., Halpin, C., Jez, J.M., Kough, J., Parrott, W., Underhill, L., Weber, N*., et al.* (2013). Evaluating the potential for adverse interactions within genetically engineered breeding stacks. Plant Physiology *161*, 1587-1594.

Swanson, D., Block, R., and Mousa, S.A. (2012). Omega-3 Fatty Acids EPA and DHA: health benefits throughout life. Advances in Nutrition *3*, 1-7.

Tracey, J., Bomford, M., Hart, Q., Saunders, G., and Sinclair, R. (2007). Managing bird damage to fruit and other horticultural crops. (Bureau of Rural Sciences, Australian Government).

Twigg, L.E., Lowe, T.J., Taylor, C.M., Calver, M.C., Martin, G.R., Stevenson, C., and How, R. (2009). The potential of seed-eating birds to spread viable seeds of weeds and other undesirable plants. Austral Ecology *34*, 805-820.

Twigg, L.E., Taylor, C.M., Lowe, T.J., and Calver, M.C. (2008). Can seed-eating birds spread viable canola seed? Pacific Conservation Biology *14*, 119-127.

Twining, C.W., Brenna, J.T., Lawrence, P., Shipley, J.R., Tollefson, T.N., and Winkler, D.W. (2016). Omega-3 long-chain polyunsaturated fatty acids support aerial insectivore performance more than food quantity. Proceedings of the National Academy of Sciences *113*, 10920-10925.

Vodkin, L.O., Rhodes, P.R., and Goldberg, R.B. (1983). cA lectin gene insertion has the structural features of a transposable element. Cell *34*, 1023-1031.

Walton, G., Mendham, M., Robertson, M., and Potter, T. (1999). Phenology, physiology and agronomy. In Canola in Australia: the first thirty years, P. Salisbury, T. Potter, G. McDonald, and A.G. Green, eds. (10th International Rapeseed Congress Organising Committee), pp. 9-14.

Wei, W., and Darmency, H. (2008). Gene flow hampered by low seed size of hybrids between oilseed rape and five wild relatives. Seed Science Research *18*, 115-123.

Wheaton, D.H., Hoffman, D.R., Locke, K.G., Watkins, R.B., and Birch, D.G. (2003). Biological safety assessment of docosahexaenoic acid supplementation in a randomized clinical trial for X-linked retinitis pigmentosa. Arch Ophthalmol *121*, 1269-1278.

Whelan, J. (2009). Dietary stearidonic acid is a long chain (n-3) polyunsaturated fatty acid with potential health benefits. Journal of Nutrition *139*, 5-10.

Yoshimura, Y., Beckie, H.J., and Matsuo, K. (2006). Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. Environmental biosafety research *5*, 67-75.

Zhang, Q., Yu, R., Sun, D., Bai, Z., Li, H., Xue, L., Zhang, Y.*, et al.* (2017). PrLPAAT4, a putative Lysophosphatidic Acid Acyltransferase from *Paeonia rockii,* plays an important role in seed fatty acid biosynthesis. Molecules (Basel, Switzerland) *22*.

Zhang, X., Li, M., Wei, D., and Xing, L. (2008). Identification and characterization of a novel yeast omega3-fatty acid desaturase acting on long-chain n-6 fatty acid substrates from *Pichia pastoris*. Yeast (Chichester, England) *25*, 21-27.

Zhou, X.-R., Robert, S.S., Petrie, J.R., Frampton, D.M.F., Mansour, M.P., Blackburn, S.I., Nichols, P.D.*, et al.* (2007). Isolation and characterization of genes from the marine microalga *Pavlova salina* encoding three front-end desaturases involved in docosahexaenoic acid biosynthesis. Phytochemistry *68*, 785-796.

Zubair, M., and Khalique, M. (2016). Review on effects of dietary poly unsaturated fatty acids on semen quality of birds and mammals, Vol 2016.

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

The Regulator received a number of submissions from prescribed experts, agencies and authorities[6] 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	Comment
1	No comment regarding the proposed trial.	Noted.
2	The invitation to comment was referred to their Pest Management Team.	Noted. No further comments were received after this email.
3	Notes licence will prohibit use of GM material in human food or animal feed. No further comments.	Noted.
4	Concern related to any 'untoward effect' due to the wide geographical spread across the intended sites	The RARMP has concluded that risks from the trial can be managed to protect human health and safety and the environment. All field trial sites are subject to strict limits and controls. The licence imposes various conditions to restrict dispersal of GM plants, seed or pollen from the trial sites. Licence holders are responsible for the management of the GM canola in field trials and must comply with conditions in the licence. Criminal penalties apply for non-compliance with licence conditions.
	Asked for clarification that a thorough risk assessment had been conducted on the herbicide tolerance gene.	A thorough risk assessment regarding the herbicide tolerance gene has been undertaken. The gene was assessed as posing negligible risk to human or animal health or to the environment. Since the identity of the herbicide tolerance gene has been declared CCI under section 185 of the *Gene Technology Act 2000* (the Act), the details regarding the assessment are addressed in the CCI attachment to the RARMP. The relevant CCI was offered for consideration as required by the Act.
5	Agrees with the overall conclusions of the RARMP.	Noted.
	Suggests further discussion of potential increased fitness in pest species, including birds, that feed on the GM canola seed (Risk scenario 2), including availability of other food sources in Queensland planting areas. Suggests that the RARMP should consider additional reports on benefits of fatty acid consumption.	Text has been added to Risk scenario 2, where relevant, to address the possible effects of the GM canola on pest species and uncertainty identified due to the contradictory results in the literature. The scenario was also amended to include the alternative food sources for pests in Queensland. Risk scenario 2 concluded that potential harm to the environment through increased fitness of pests, including birds, was negligible. The text has been amended in Chapter 2 section 3 and Chapter 3 section 4 of the RARMP to address uncertainty regarding the potential advantage for pests as a result of feeding on the GM canola.

[6] Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment and Energy.

Submission	Summary of issues raised	Comment
	Agrees with the conclusion that the risks of environmental harm resulting from pollen-mediated gene flow to weedy relatives is negligible, but suggests further discussion of potential seed-mediated dispersal, especially by birds. Suggests including additional discussion whether the traits could increase fitness in the parent or in hybrids with weedy relatives.	Noted. Text has been added to Risk scenario 3, where relevant, to address potential endozoochory. Risk scenario 3 concluded that increased weediness of the GMOs was not a substantive risk. Text has been added to risk scenario 4 to address likelihood of hybrids being produced. Risks associated with GM hybrids are unlikely to differ from those posed by GM canola plants, and therefore are considered similarly negligible.
6	Agrees that the RARMP adequately identifies and manages the risks that would be of local concern, such as pollen drift to non-GM crops.	Noted.
7	Satisfied with the overall RARMP conclusions and with the measures taken to manage the short and term long risks of the application.	Noted.
8	Asks if the proposed scale plantings are acceptable for a limited and controlled trial, and if the exact LGA location can be found.	After considering information supplied in the application, the Regulator made the decision, prior to preparing the RARMP, that the application satisfied the requirements for a limited and control release in accordance with section 50A of the Act. Apart from listing the LGAs, the exact site locations have not yet been decided. The risk assessment considers characteristics of the broad geographic regions where the release is proposed to occur. Once GMOs are planted at a site, GPS coordinates will be posted on the OGTR website.
	Asks for clarification on the animal feeding experiments, including the location where they will be undertaken.	Institutional oversight of experimental use of animals is by animal ethics committees, which operate under *The Australian Code for the Care and Use of Animals for Scientific Purposes*. For detail of the type of animal feeding experiments that are envisaged, see Chapter 2 of the RARMP. Exact locations of these are currently not determined.
	Concerns regarding how the GM canola will perform on varying biotic and abiotic environmental stresses in comparison to baseline canola.	The RARMP assesses the controls proposed by the applicant for the field trial and has concluded that these are suitable to restrict the trial. Some of the GM canola lines described in this application have not been grown in the field before. The aim of this field trial is to gather information regarding the agronomic performance of GM canola under field conditions, as well information needed for future regulatory approvals.
	Asks for further clarification on the information regarding the international approvals.	Text has been added where appropriate to clarify the information regarding international releases.
9	Asks if the 150 hectares per year for 5 years is really required to undertake the research. Asks if a consideration been made in reducing the area and thus reducing the risk of dispersal.	After considering information supplied in the application, the Regulator made the decision, prior to preparing the RARMP, that the application satisfied the requirements for a limited and controlled release in accordance with section 50A of the Act. The likelihood of dispersal of the GM canola into the environment through several pathways was assessed in Chapter 2 of the RARMP. These pathways were found to be unlikely, taking into account the biology of the species, the location of the trial and the limits and controls imposed on the release that will prevent the dispersal of the GM canola

Submission	Summary of issues raised	Comment
		from the trial site and its persistence after the trial.
	Asks if the proposed 50 metre monitoring zone and up to one kilometre isolation zone are sufficient to detect and prevent pollen dispersal. Asks if two years of post-harvest monitoring are sufficient enough to address the secondary dormancy of canola. Asks how effective the tillage and irrigation post-harvest are in addressing the secondary dormancy.	Strict licence conditions have been imposed to minimise spread and persistence of the GM canola and the introduced genetic material in the environment. Based on current information and experience, the control measures imposed by the licence are effective for restricting spread and persistence of the GM crop. The likelihood of pollen dispersal from the GM canola into the environment through several pathways was evaluated in Chapter 2 Risk scenario 4 of the RARMP, and the suitability of controls assessed in Chapter 3 in the RARMP for DIR 123, which used the same controls. These controls have been effective for previous field trials. Canola is predominantly self-pollinated and the majority of cross-pollination occurs within a 4.5 - 10 m area around the GM pollen source. Under Australian conditions, a large scale field study found that gene flow between plants at 30 m separation was reported to be 0.03% (see RARMP Chapter 1, section 6). The potential for pollen flow from the field trial is therefore considered highly unlikely when the combination of 50 m Monitoring Zone free of related species and 1 km Isolation Zone free of deliberately planted canola crops is employed. Regarding post-harvest monitoring, the likelihood of dispersal of GM seeds outside the trial limits was evaluated in Chapter 2 Risk scenario 3, concluding this risk not to be greater than negligible. The majority of volunteer seedlings emerge the year following a canola crop. The licence imposes a two-year minimum post-harvest monitoring as long as no volunteers (unintentionally grown canola plants) are detected in the area for at least the last 12 months. If canola volunteers are detected within the last 12 months of inspection, then the inspection period is extended until 12 months of inspections find the area free of volunteers. In combination with inspection requirements, there are tillage and irrigation requirements to bring seed to the surface and encourage germination. Further information regarding the suitability of this control can be found in the risk management plan for DIR 123 (Chapter 3, paragraph 156). The promotion of seed bank germination for GM canola was discussed in a previous canola RARMP, DIR 114. Tillage must not bury plant material to a depth of more than sowing and ideally would occur in conditions where germination of the GMO is reasonably likely to ensue (e.g. after irrigation or rainfall). These treatments would promote germination by ensuring any remaining seeds are placed at an appropriate depth in conditions that promote germination and will also encourage the microbial decomposition of any residual seed.
	Comments that a higher content of PUFA in GM canola could lead to better drought tolerance. Suggests that the OGTR should confirm the drought tolerance of the GM canola before the field trials.	Some of the GM canola lines described in this application have not been grown in the field before. The aim of this field trial is to gather information regarding the agronomic performance of GM canola under field conditions, as well information needed for future regulatory approvals. This will include drought tolerance and other biotic and abiotic stresses.

Submission	Summary of issues raised	Comment
	Asks if there has been any consultation with non-GM canola growers in the 95 local government areas earmarked for field trials.	As required by the Act, the Regulator invited comments on the consultation RARMP from the general public, relevant Australian local councils, prescribed experts, Australian Government authorities and agencies, State and Territory Governments and the Minister for the Environment. This was via ads in *The Land* (rural NSW), *Stock and Land* (rural VIC) and in *Queensland Country Life* (rural QLD) newspapers, a notice on the OGTR website, in the Australian government gazette and by tweets from the Department of Health Twitter account.
	Asks if the applicant will seek ethics approval prior to undertaking sensory testing of the oil. Wants any adverse reactions to be documented in the study and provided to the Regulator and health agencies.	Yes. As clarified in the RARMP, limited sensory oil tasting would be carried out subject to oversight by a Human Research Ethics Committee, which is required to review and approve the research proposals in accordance with the National Statement on Ethical Conduct in Human Research. Additionally, the licence holder must inform the Regulator of additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence.
	Asks if there have been any studies undertaken by the applicant comparing the allergenicity of GM and non-GM canola.	Information regarding the toxicity and allergenicity of the proteins and end products associated with the introduced genes has been addressed in Chapter 1 of the RARMP.
	Considers that the competitive edge that the GM seeds with altered oil profile would provide to a pest should be addressed in a larger context rather than the current application. Wants the Regulator to consider if only GM canola was grown that this would provide nutritional advantage to certain pests and reduce biodiversity.	The possible effects of the GM canola on pest species are addressed in Chapter 2 of the RARMP, Risk scenario 2. This risk scenario concluded that potential harm to the environment through increased fitness of pests, including birds, was negligible. The text has been amended in Chapter 2 section 3 and Chapter 3 section 4 of the RARMP to address uncertainty regarding the potential advantage for pests as a result of feeding on the GM canola.
	Requests to receive information on the CCI genes.	The relevant CCI was made available as required by the Act. No revised comments were received after the CCI had been provided.
10	Agrees with the overall conclusions of the RARMP and did not require additional relevant information.	Noted.

Appendix B: 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 the 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	Comment
1	Concerned about the development of herbicide resistance as a result of modifying crops with herbicide tolerance, and the implications in weed and GM crop management for farmers in the South West of Western Australia.	Herbicide resistance issues come under the regulatory oversight of the Australian Pesticides and Veterinary Medicines Authority (APVMA). Discussion of Integrated Weed Management, which is designed to limit the development of herbicide resistance in weed populations, is included in section 2 of chapter 2 of the Risk Assessment and Risk Management Plan (RARMP).
2	Is opposed to genetic modification.	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 for each licence application received. This submission is outside the Regulator's legislative responsibility.
3	Is opposed to the use of glyphosate resistant plants. Refers to studies done in the US, Canada and underway in Australia regarding levels of glyphosate residues in food products.	The Australian Pesticides and Veterinary Medicines Authority (APVMA) has regulatory responsibility for agricultural chemicals, including herbicides, in Australia. See the APVMA website for further information. APVMA and Food Standards Australia New Zealand (FSANZ) have shared responsibilities in setting maximum residue limits for agricultural chemicals in food. The FSANZ website has an information page on herbicides in GM foods.
4	Does not believe the risk assessment to be accurate if you look at history and the poisoning of the food chain simply to increase profits. Suggests we do not care about the Australian eco system and people's health.	The Regulator is required to evaluate licence applications in accordance with the *Gene Technology Act 2000*, the object of which is to protect the health and safety of people and the environment posed by or as a result of gene technology. For each licence application, the Regulator must prepare a risk assessment and risk management plan (RARMP) in accordance with the Act and the *Gene Technology Regulations 2001* prior to making a decision whether or not to issue a licence. For details of the OGTR approach to risk analysis, please refer to Risk Analysis Framework 2013. The RARMP for DIR 163 includes consideration of the following: • harm to the health of people or desirable organisms, including toxicity/allergenicity • reduced biodiversity • 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 (e.g. providing food or shelter for pests or pathogens) or abiotic environment (e.g. negative effects on fire regimes, nutrient levels, soil salinity, soil stability or soil water

Submission	Summary of issues raised	Comment
		table). The RARMP prepared for each application for a licence to grow a GM crop includes a thorough and critical assessment of data supplied by the applicant, as well as a comprehensive review of other relevant national and international scientific literature. Scientific and other literature is monitored for any new information relevant to GMOs and GM foods, and assessed for its potential to impact on the health and safety of people and the environment. Prior to finalising the RARMP and making a decision on the licence, the Regulator consults with a broad range of experts and authorities, including the Gene Technology Technical Advisory Committee (GTTAC), the Minister for the Environment, all State and Territory Governments and relevant local councils. In addition, the public is consulted through invitations to comment published in national and local newspapers and on the OGTR website. The Regulator's evaluation concluded that this release poses negligible risk to human health or the environment.
	Is opposed to planting GM anywhere in Australia.	Matters related to consumer preferences are outside the Regulator's legislative responsibility.
	Suggests looking at the health implications	This application is for a limited and controlled release (field trial), a licence condition has been imposed to prohibit GM plant material or products being used for human food or animal feed. While the Regulator must consider risks to human health and safety and the environment relating to dealings with GMOs, other agencies have responsibility for regulating GMOs or genetically modified products as part of a broader or different mandate. FSANZ is responsible for human food safety assessment. Further information on the regulation, assessment and labelling of GM foods is available from the FSANZ website.
5	Is concerned about the potential spread of the GM canola, the likelihood that it could persist and out-compete other varieties, and the potential of the genetic modification to persist across generations.	The current licence authorises a field trial of GM canola. As required by the Act and Regulations, the Regulator has prepared a RARMP which addresses these concerns as well as any risks to human health and safety, and risks to the environment from this field trial. The likelihood of an escape of the GM canola into the environment through several pathways was assessed in chapter 2 of the RARMP, and the risk scenario was not identified as a substantive risk. Strict licence conditions have been imposed to minimise spread and persistence of the GM canola and the introduced genetic material in the environment. Based on current information and experience, the control measures are considered to be effective for restricting the spread of the GM canola.
	Asks if the Regulator can tell apart the authorised GM canola from other canola.	A testing methodology for the unique identification of the GM canola must be made available to the Regulator before any authorised dealings can be commenced.
	Asks about labelling of food products derived from GM crops.	Labelling of food, including GM foods, is the responsibility of FSANZ. Further information on the regulation, assessment and labelling of GM foods is available from the FSANZ website. However, as this application is for a limited and controlled release (field trial), a licence condition has been imposed to prohibit GM plant material or products being used for

Submission	Summary of issues raised	Comment
		commercial human food or animal feed.
	Asks about financial consequences in case the GM canola spreads outside the field trial, whether the company should be bonded to cover any risk or cost, e.g. Who will pay for any clean-up in case adverse effects are observed? Will a non-GM farmer have to pay royalties to the patent holder? Will it impact on the value of an organic crop?	This licence authorises a field trial (limited and controlled release) of GM canola. Strict licence conditions have been imposed to minimise spread and persistence of the GM canola and the introduced genetic material in the environment. Prior to licence issue, the Regulator considered the suitability of Nuseed to hold the licence. The licence holder is responsible for the control and clean-up of any spread or spill of GM material outside the trial sites. Prior to conducting any dealings, the licence holder is required to prepare a contingency plan for measures to be taken in the event of the unintended presence of the GMOs outside an area that must be inspected. If suspected that GM plant material has dispersed from the trial site then this must be reported to the Regulator immediately. The Regulator may then direct the licence holder to commence any necessary steps for mitigation of any risk and can vary, suspend or cancel the licence. Criminal penalties apply for non-compliance with licence conditions.
	Asks for a cost/risk/benefit estimate of the genetic modification.	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. Economic risks lie outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence. The Regulator cannot consider economic or other benefits.
	Asks how Australia controls the introduction of GMOs from other countries with few regulations and little oversight.	Biosecurity requirements both for non-GM organisms and GMOs are the responsibility of the Department of Agriculture. However, in the case of GMOs, all dealings with GMOs also require an authorisation from the Regulator. This includes import, transport, creating and growing GMOs. The Act provides for substantive penalties in case of non-compliance.
	Believes Australia needs a genomic test range, possibly in cooperation with other governments and experts, to avoid or defend against deliberate attempts to bypass regulation in order to create novel bio-engineered organisms (biohacking).	These matters are outside of the scope of the considerations relevant for this field trial for GM canola.
	Is concerned about the potential spread of the GM canola, the likelihood that it could persist and out-compete other varieties, and the potential of the genetic modification to persist across generations.	The current licence authorises a field trial of GM canola. As required by the Act and Regulations, the Regulator has prepared a RARMP which addresses these concerns as well as any risks to human health and safety, and risks to the environment from this field trial. The likelihood of an escape of the GM canola into the environment through several pathways was assessed in chapter 2 of the RARMP, and the risk scenario was not identified as a substantive risk. Strict licence conditions have been imposed to minimise spread and persistence of the GM canola and the introduced genetic material in the environment. Based on current information and experience, the control measures are considered to be effective for restricting the spread of the GM canola.