Australian Government
Department of Health
Office of the Gene Technology Regulator

June 2022

Risk Assessment and Risk Management Plan for

DIR 188

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

Applicant: Nuseed Pty Ltd

June 2022

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Summary of the Risk Assessment and Risk Management Plan for Licence Application No. DIR 188

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 has been prepared by the Regulator in accordance with 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 proposed 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

Applicant	Nuseed Pty Ltd
Project title	Limited and controlled release of canola and Indian mustard genetically modified for altered oil content and herbicide tolerance
Parent organisms	Canola (*Brassica napus* L.) Indian mustard (*Brassica juncea* (L.) Czern. & Coss.)
Introduced genes	Seven genes involved in biosynthesis pathway for long-chain polyunsaturated fatty acids: • *Lackl-Δ12D* from yeast *Lachancea kluyveri* • *Picpa- ω3D* from yeast *Pichia pastoris* • *Micpu-Δ6D* from microalga *Micromonas pusilla* • *Pyrco-Δ6E* from microalga *Pyramimonas cordata* • *Pavsa-Δ5D* from microalga *Pavlova salina* • *Pyrco-Δ5E* from microalga *Pyramimonas cordata* • *Pavsa-Δ4D* from microalga *Pavlova salina* One gene that confers herbicide tolerance: • *pat* gene from soil bacterium *Streptomyces viridochromogenes* for glufosinate tolerance
Proposed locations	Up to 20 trial sites per year to be selected from 96 possible local government areas in New South Wales, Victoria and Queensland
Proposed release size	Up to 150 ha per year
Proposed period of release	From November 2022 to January 2028
Principal purpose	To evaluate the altered oil content trait under field conditions

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.

Credible pathways to potential harm that were considered included exposure of people or other desirable organisms to the GM plant material, potential for persistence or dispersal of the GMOs, and transfer of the introduced genetic material to canola, Indian mustard, and related plants outside the field trial. Potential harms associated with these pathways included toxicity or allergenicity to people, toxicity to other desirable organisms, and environmental harms due to weediness.

The principal reasons for the conclusion of negligible risks are that the proposed limits and controls will effectively minimise exposure to the GMOs, and there is no evidence to suggest the introduced genetic modifications would lead to harm to people or the environment.

Risk management

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 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 sites, to transport the GMOs in accordance with the Regulator's guidelines, to destroy GMOs at the end of the trial and to conduct post-harvest monitoring at the trial sites to ensure the GMOs are destroyed.

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Abbreviations

ALA	α-linolenic acid
APVMA	The Australian Pesticides and Veterinary Medicines Authority
CCI	Confidential Commercial Information
DIR	Dealings involving Intentional Release
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
ETA	Eicosatetraenoic acid
FSANZ	Food Standards Australia New Zealand
GM(O)	Genetically modified (organism)
ha	Hectare(s)
HGT	Horizontal gene transfer
km	Kilometre(s)
LC-PUFA	Long chain polyunsaturated fatty acid
m	Metre(s)
mm	Millimetre(s)
NSW	New South Wales
OGTR	Office of the Gene Technology Regulator
PAT	Phosphinothricin N-acetyltransferase
PUFA	Polyunsaturated fatty acid
RARMP	Risk Assessment and Risk Management Plan
the Regulations	The Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
the Act	The *Gene Technology Act 2000*
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 and the Gene Technology Regulations 2001 (the Regulations), together with corresponding State and Territory legislation, 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. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and Sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.

4. The *Risk Analysis Framework* (OGTR, 2013a) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator (OGTR) website.

5. Figure 1 shows the information that is considered, within the regulatory framework, in establishing the risk assessment context. This information is specific for each application. Risks to the health and safety of people or the environment posed by the proposed release are assessed within this context. Chapter 1 provides the specific information for establishing the risk assessment context for this application.

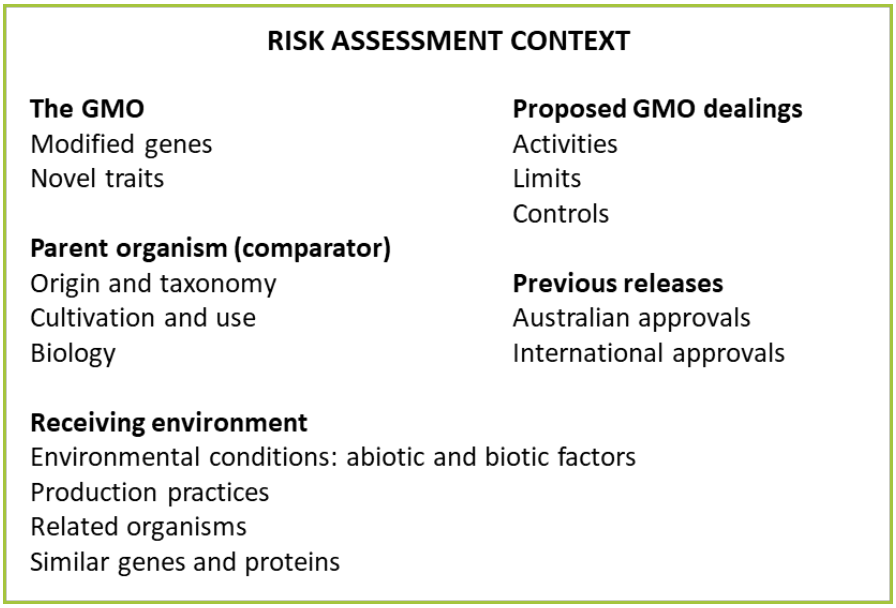

Figure 1. Summary of parameters used to establish the risk assessment context

6. Section 52 of the Act requires the Regulator to seek comment on the RARMP from agencies - the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, Australian local councils and the Minister for the Environment - and from the public. The advice from the prescribed experts, agencies and

authorities and how it was taken into account is summarised in Appendix A. One public submission was received and its consideration is summarised in Appendix B.

7. The GMOs and any proposed dealings 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 (APVMA), the Therapeutic Goods Administration and the Department of Agriculture, Water and the Environment. Proposed dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

Section 2 The proposed dealings

8. Nuseed Pty Ltd (Nuseed) proposes to release up to 80 GM canola and Indian mustard lines into the environment under limited and controlled conditions. The GM plants have been genetically modified for altered seed oil content and herbicide tolerance.

9. The purpose of the release is to evaluate the altered oil content trait under field conditions. The field trial will gather research and regulatory data about agronomic performance, oil content and profile, nutritional assessment, compositional analysis, molecular analysis, and genetic stability.

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 the possession, supply or use of the GMOs in the course of any of these dealings.

11. GM plant material would not be used in commercial human food or animal feed.

12. GM plant material may be exported and used in a human nutritional study outside Australia. Proposed dealings with GM plant material in countries other than Australia do not fall within the jurisdiction of Australia's Gene Technology Regulator and will not be considered in this RARMP.

13. GM plant material may be used in human sensory testing to assess the feel, smell, taste and appearance of the seed oil or food products containing the oil. Sensory testing would result in negligible consumption of the oil as the products are not intended to be swallowed during testing. These trials would only occur if Nuseed has the appropriate approvals for each trial in accordance with the National Statement on Ethical Conduct in Human Research.

14. GM plant material may be used in animal feeding studies. These could include toxicology trials with rodents, bioavailability trials with rodents, broiler chicken feeding trials and aquaculture feeding trials. These trials would only occur if Nuseed has the appropriate approvals for each trial in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes.

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

15. The field trial is proposed to take place over five years, between November 2022 and January 2028. In each year there would be up to ten trial sites of 10 ha and ten trial sites of 5 ha, with a total trial area of up to 150 ha per year.

16. The trial sites would be selected from 96 local government areas in New South Wales (NSW), Victoria and Queensland (Table 1). The trial sites would be located on private land in rural areas. Details of site locations would be provided to the Regulator prior to each planting season.

Table 1. Local government areas where proposed trial sites may be located

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	Somerset Regional Council
Bland Shire Council	Buloke Shire Council	Southern Downs Regional Council
Blayney Shire Council	Campaspe Shire Council	Toowoomba Regional Council
Cabonne Shire Council	Central Goldfields Shire Council	Western Downs Regional Council
Carrathool Shire Council	Colac-Otway Shire Council	
Coolamon Shire Council	Corangamite Shire Council	
Coonamble Shire Council	Gannawarra Shire Council	
Cootamundra- Gundagai Regional Council	Glenelg Shire Council	
Cowra Shire Council	Golden Plains Shire Council	
Dubbo Regional Council	Greater Bendigo City Council	
Edward River Council	Greater Geelong City Council	
Federation Council	Greater Shepparton City Council	
Forbes Shire Council	Hepburn Shire Council	
Gilgandra Shire Council	Hindmarsh Shire Council	
Greater Hume 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	
Hilltops Council	Melton Shire Council	
Junee Shire Council	Mildura Rural City Council	
Lachlan Shire Council	Mitchell Shire Council	
Leeton Shire Council	Moira Shire Council	
Liverpool Plains Shire Council	Moorabool Shire Council	
Lockhart Shire Council	Mount Alexander Shire Council	
Mid-Western Regional Council	Moyne Shire Council	
Moree Plains Shire Council	Murrindindi Shire Council	
Murray River Council	Northern Grampians Shire Council	
Murrumbidgee Council	Pyrenees Shire Council	
Muswellbrook Shire Council	South Gippsland Shire Council	
Narrabri Shire Council	Southern Grampians Shire Council	
Narrandera Shire Council	Strathbogie Shire Council	
Narromine Shire Council	Surf Coast Shire Council	

New South Wales	Victoria	Queensland
Orange City Council	Swan Hill Rural City Council	
Parkes Shire Council	Towong Shire Council	
Snowy Valleys Council	Wangaratta Rural City Council	
Tamworth Regional Council	Warrnambool City Council	
Temora Shire Council	Wellington Shire Council	
Upper Hunter Shire Council	West Wimmera Shire Council	
Wagga Wagga City Council	Wodonga City Council	
Walgett Shire Council	Wyndham City Council	
Warren Shire Council	Yarriambiack Shire Council	
Warrumbungle Shire Council		
Weddin Shire Council		

17. Only trained and authorised staff would be permitted to deal with the GM plants.

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

18. The applicant has proposed a number of controls to restrict the spread and persistence of the GM canola and Indian mustard and the introduced genetic material in the environment. These include:

- locating each proposed trial site at least 50 m away from the nearest natural waterway
- restricting gene flow from the GMOs using one of the combinations of controls shown in Figure 2
- treating any non-GM canola or Indian mustard plants grown in planting areas or pollen traps like the GMOs
- harvesting the GMOs separately from other crops
- after harvest, destroying GMOs not required for further evaluation or future trials
- cleaning equipment used in connection with the GMOs as soon as practicable and before use for any other purpose
- transporting and storing GMOs in accordance with the current Regulator's Guidelines for the Transport, Storage and Disposal of GMOs
- post-harvest tilling of planting areas, pollen traps and other areas where GMOs were dispersed to encourage seed germination
- post-harvest monitoring of each trial site monthly for at least 2 years and until the site is free of volunteer canola or Indian mustard plants for at least 12 months, with any volunteer plants destroyed prior to flowering
- during the post-harvest monitoring period, planting only crops permitted on GM brassica trial sites by the Regulator's Policy on Post-Harvest Crops.

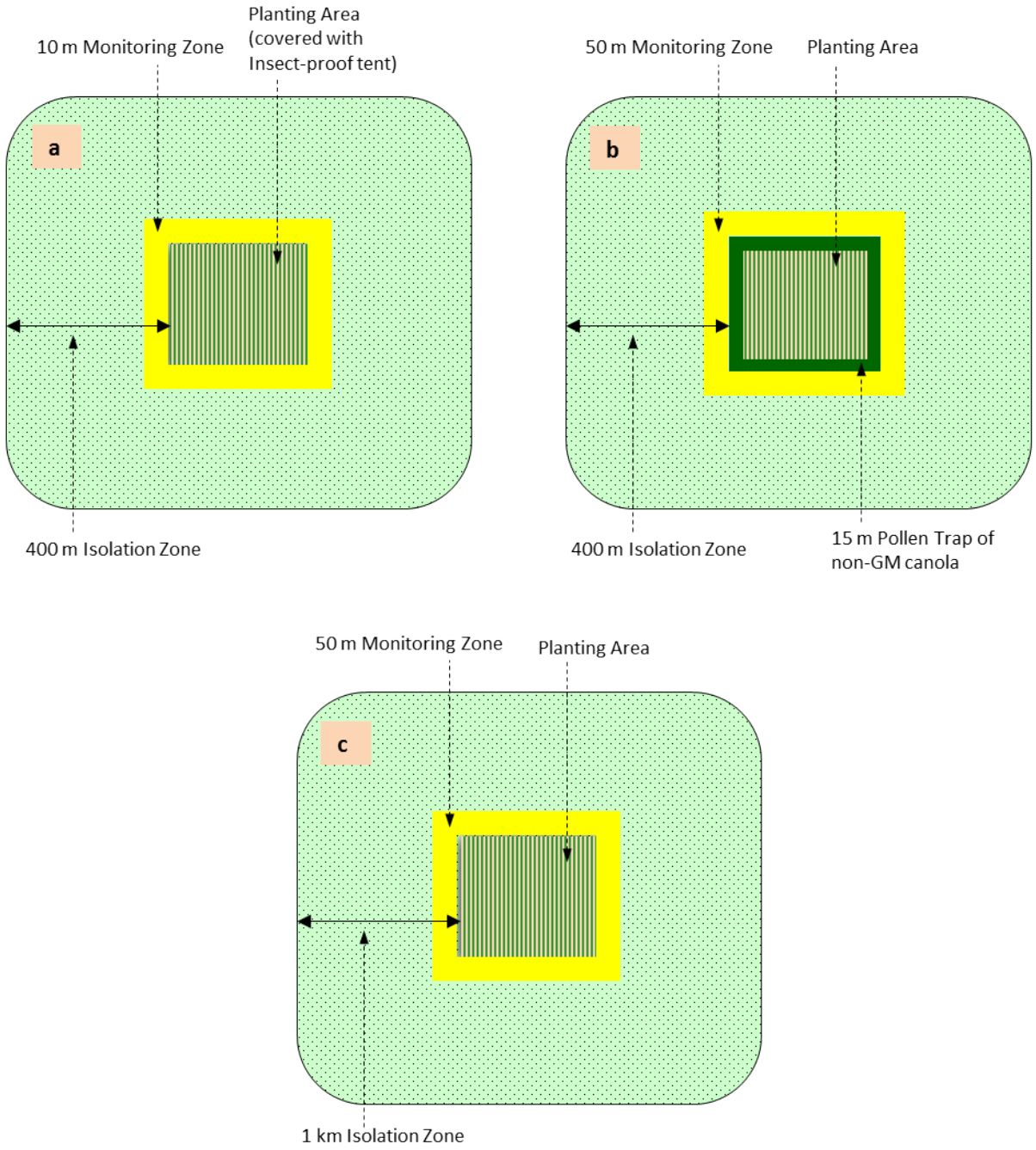

Figure 2. Options for restricting gene flow from the GM canola and Indian mustard (not to scale). Site layout (a) with Insect-proof tent covering the GMOs during flowering, (b) without Insect-proof tent and with Pollen Trap surrounding the Planting Area, and (c) without Insect-proof tent or Pollen Trap. Monitoring and Isolation Zones must be kept free of related plants.

19. The proposed limits and controls are taken into account in the risk assessment (Chapter 2) and their suitability for containing the release will be evaluated in the risk management plan (Chapter 3).

Section 3 The parent organism

20. The parent organisms are *Brassica napus* L., which is commonly known as canola, rapeseed or oilseed rape, and *Brassica juncea* (L.) Czern. & Coss., which is commonly known as Indian mustard or juncea canola. *B. napus* and *B. juncea* are both exotic to Australia.

21. Canola is the third-most widely grown crop in Australia. It is grown mainly in Western Australia, NSW, Victoria and South Australia (ABARES, 2021). Canola oil is used as food and the canola meal remaining

after oil extraction is used as animal feed. Almost all commercial canola grown in Australia is *B. napus*, but a small amount is canola-quality *B. juncea*, which is adapted to low-rainfall areas. Other varieties of *B. juncea* are grown in Australia to produce condiment mustard (GRDC, 2017). The GM Indian mustard proposed for release is derived from canola-quality *B. juncea* varieties.

22. Both *B. napus* and *B. juncea* are naturalised in Australia. In areas where they are grown, they can be agricultural weeds in subsequent crops. There are isolated reports of *B. napus* as an environmental weed in Western Australia and *B. napus* and *B. juncea* as environmental weeds in Victoria (Randall, 2017). However, the most recent Western Australian state government environmental weed risk assessment gives *B. napus* a weed risk rating of negligible (Environmental weed risk assessments, accessed 10 Jan 2022), and the most recent Victorian state government environmental weed list gives both *B. napus* and *B. juncea* risk ranking scores of zero (White et al., 2018).

23. Detailed information about the parent organisms is contained in the document *The Biology of Brassica napus L. (canola) and Brassica juncea (L.) Czern. & Coss. (Indian mustard)* (OGTR, 2017), which was produced to inform the risk analysis process and is available from the Resources page on the OGTR website. Baseline information from this document will be used and referred to throughout the RARMP.

24. Some information about the specific parent organisms used for this application has been declared Confidential Commercial Information (CCI). Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

Section 4 The GMOs, nature and effect of the genetic modification

25. The applicant proposes to grow up to 80 lines of GM canola and Indian mustard with altered oil content and herbicide tolerance. Some information about the categories of GMOs proposed for release has been declared Confidential Commercial Information (CCI). Under Section 185 of the Act, the confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

4.1 The genetic modifications in the GMOs proposed for release

26. The GMOs contain up to seven introduced genes involved in fatty acid biosynthesis and one introduced gene that confers herbicide tolerance.

27. The seven introduced fatty acid biosynthesis genes (Table 2) were sourced from yeast and marine microalgae, and codon optimised for expression in higher plants.

Table 2. Introduced genes involved in fatty acid biosynthesis

Gene	Source organism	Encoded protein	Reference
Lackl-Δ12D	*Lachancea kluyveri* yeast	Δ12-desaturase	(Petrie et al., 2012)
Picpa-ω3D	*Pichia pastoris* yeast	ω-3 desaturase	(Zhang et al., 2008)
Micpu-Δ6D	*Micromonas pusilla* microalgae	Δ6-desaturase	(Petrie et al., 2010b)
Pyrco-Δ6E	*Pyramimonas cordata* microalgae	Δ6-elongase	(Petrie et al., 2010a)
Pavsa-Δ5D	*Pavlova salina* microalgae	Δ5-desaturase	(Zhou et al., 2007)
Pyrco-Δ5E	*Pyramimonas cordata* microalgae	Δ5-elongase	(Petrie et al., 2010a)
Pavsa-Δ4D	*Pavlova salina* microalgae	Δ4-desaturase	(Zhou et al., 2007)

28. The purpose of the introduced fatty acid biosynthesis genes is to convert oleic acid, which is an abundant fatty acid in canola and Indian mustard seed oil, into ω-3 long-chain polyunsaturated fatty acids (LC-PUFAs), which are not naturally present in plant seed oil (Ruiz-Lopez et al., 2015; Saini et al., 2021). ω-3 LC-PUFAs are fatty acids of 20 or more carbons in length with multiple cis double bonds in their backbone,

with the first double bond on the third carbon from the methyl end. The fatty acid biosynthesis pathways are shown in Figure 3.

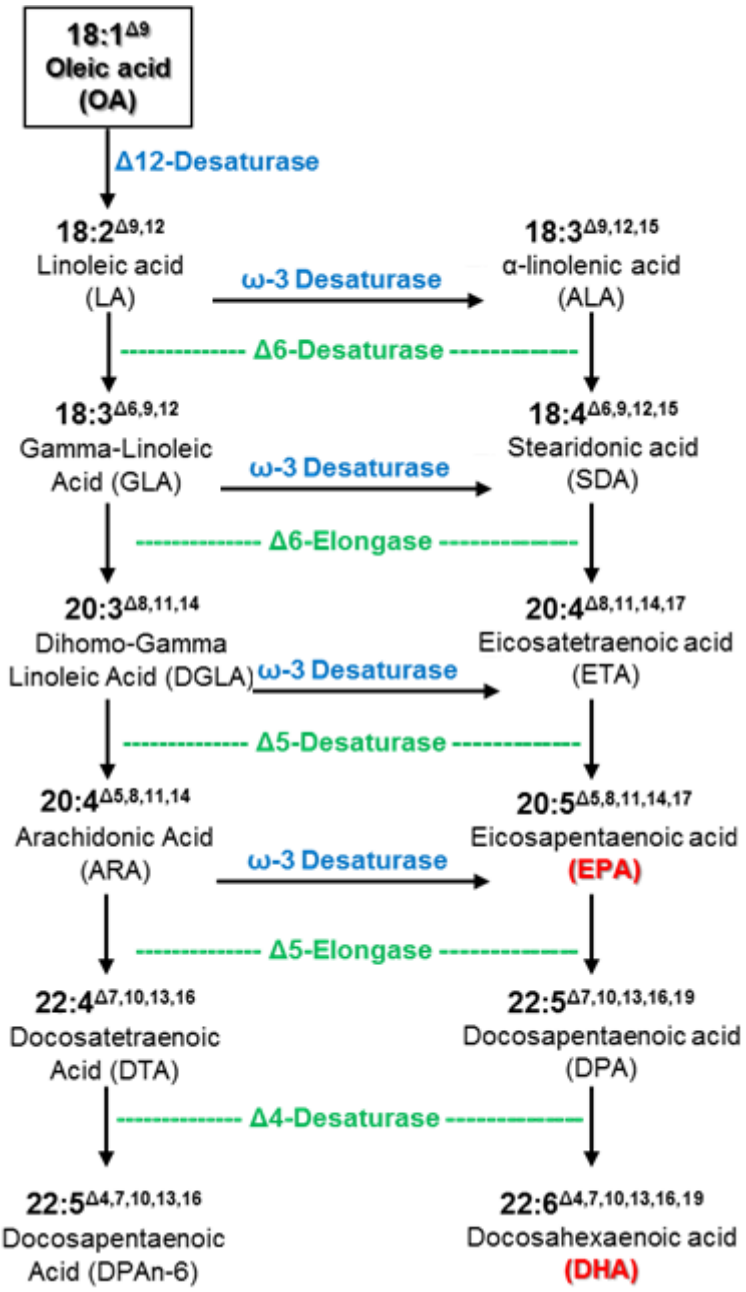

Figure 3. Outline of pathways for biosynthesis of ω-3 LC-PUFAs in GM plants, adapted from Ruiz-Lopez et al. (2013). The main ω-3 LC-PUFAs with importance for human health are highlighted in red.

29. The *Picpa-ω3D* ω-3 desaturase introduced into the GMOs has similar conversion rates for all 18-carbon and 20-carbon substrates (Zhang et al., 2008). However, the right-most biosynthesis pathway in the diagram is likely to be preferred in the GMOs, as Δ6-desaturation is reported to be the rate-limiting step (Petrie et al., 2020) and the *Micpu-Δ6D* Δ6-desaturase has 3.5-fold greater conversion efficiency with an α-linolenic acid (ALA) substrate than with a linoleic acid (LA) substrate (Petrie et al., 2010b).

30. The GMOs may also contain the *pat* gene. The *pat* gene is sourced from the soil bacterium *Streptomyces viridochromogenes.* It encodes the phosphinothricin N-acetyltransferase (PAT) enzyme, which confers tolerance to glufosinate (phosphinothricin) herbicide. The *pat* gene was used as a selectable marker

gene during initial development of the GM plants in the laboratory to select plant cells containing the introduced genes and glufosinate herbicide may also be used as a crop management tool in the field trial.

31. Short regulatory sequences that control gene expression have also been introduced into the GMOs (Table 3). The expression of the introduced fatty acid biosynthesis genes is targeted to the seed with seed-specific promoters, while the expression of the *pat* gene is driven by a constitutive promoter, which is active in all plant tissues. Other short regulatory elements used include enhancers of gene expression and terminators.

Table 3. Introduced regulatory sequences

Sequence	Source	Intended function
PRO_Arath-FAE1	Promoter of *Arabidopsis thaliana* fatty acid elongase 1	Seed specific promoter
PRO_Brana-FP1	Promoter of *Brassica napus* napin	Seed specific promoter
PRO_Linus-Cnl1	Promoter of *Linum usitatissimum* conlinin1	Seed specific promoter
PRO_Linus-Cnl2	Promoter of *Linum usitatissimum* conlinin2	Seed specific promoter
PRO_35S×2	Promoter of Cauliflower mosaic virus 35S RNA	Constitutive promoter
Tobacco mosaic virus 5' UTR leader	Enhancer from Tobacco mosaic virus 59	Increase gene expression
MAR_Nicta- RB7	Rb7 matrix attachment region from *Nicotiana tabacum*	Increase gene expression
TER_Agrtu-NOS	Terminator of *Agrobacterium tumefaciens* nopaline synthase	Terminator
TER_Glyma-Lectin	Terminator of *Glycine max* lectin *Le1*	Terminator
TER_Linus-Cnl1	Terminator of *Linum usitatissimum* conlinin1	Terminator
TER_Linus-Cnl2	Terminator of *Linum usitatissimum* conlinin2	Terminator

32. Gene constructs were introduced into the GMOs using *Agrobacterium*–mediated transformation. This method has been widely used in Australia and overseas for introducing genetic modifications into plants. More information can be found in the document *Methods of Plant Genetic Modification* which is available from the Resources page on the OGTR website.

4.2 Toxicity/allergenicity of the proteins associated with the introduced genes

33. None of the source organisms of the introduced genes are known to be toxic, allergenic or pathogenic.

34. Bioinformatic analysis may assist in the assessment process by predicting, on a theoretical basis, the toxic or allergenic potential of a protein. The sequences of the eight introduced proteins were compared to all proteins in the NCBI Entrez Protein database as well as the AllergenOnline.org database (version 18). The searches did not find any biologically relevant similarity between the introduced proteins and any known toxin or allergen (MacIntosh et al., 2021).

35. All introduced proteins were readily digested by pepsin in a standard assay of the digestibility of proteins in simulated gastric fluid (MacIntosh et al., 2021).

36. FSANZ has assessed the safety of a GM canola line containing all of the introduced genes included in this application. FSANZ concluded that food derived from the GM canola line is considered to be as safe for human consumption as food derived from conventional (non-GM) canola cultivars (FSANZ, 2017).

37. The *pat* gene and its products have been extensively characterised and assessed as posing negligible risk to human and animal health or to the environment by the Regulator, as well as by other regulatory agencies in Australia and overseas (CERA, 2011). Commercial GM canola lines containing the *pat* gene have

been assessed to pose negligible risks to human health and the environment in the RARMPs for DIR 021/2002 (OGTR, 2003), DIR 108 (OGTR, 2011) and DIR 155 (OGTR, 2018a).

38. The applicant proposes to use glufosinate herbicide as a crop management tool. The potential for toxicity of glufosinate metabolites has been considered in previous RARMPs for commercial release of GM crops including canola (DIR 021/2002, DIR 108, DIR 138, DIR 175 and DIR 178) and cotton (DIR 062/2005, DIR 143 and DIR 173). These RARMPs concluded that the main herbicide metabolites formed in GM plants following glufosinate treatment were less toxic than glufosinate and there is no suggestion that other metabolites produced by the activity of the PAT protein on endogenous plant amino acids (Christ et al., 2017) are toxic (O'Connor, 2017).

4.3 Toxicity due to the altered oil content trait

39. The GMOs are intended to produce ω-3 LC-PUFAs in seed oil. The applicant states that the GMOs will contain some or all of the fatty acid biosynthesis genes listed in Table 2. Depending on which introduced genes are present in each GM transformant, the main ω-3 LC-PUFA produced could be eicosatetraenoic acid (ETA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) or docosahexaenoic acid (DHA), as shown in Figure 3.

40. As enzymatic conversion efficiency is not 100%, the seed oil of the GMOs is expected to contain some fatty acid intermediates as well as the target ω-3 LC-PUFAs. For example, in a GM canola line containing all of the fatty acid biosynthesis genes listed in Table 2 and designed to produce a high level of DHA (Petrie et al., 2020), the fatty acid profile included 9.7% DHA, 1.0% DPA, 1.3% ETA and 2.2% stearidonic acid (SDA), while none of these fatty acids were present in the non-GM parent canola. The GM canola line was also enriched in ALA, with 20% ALA in the GM line compared to 9.5% ALA in the non-GM parent.

41. ω-3 LC-PUFAs are naturally present in seafood and are particularly abundant in oily fish. Many plant food products are rich in ALA, and human biosynthetic pathways can convert ALA into ω-3 LC-PUFAs, although at low conversion efficiencies. As ω-3 LC-PUFAs are considered to be beneficial to human health, fish oil is commonly consumed as a dietary supplement (Zarate et al., 2017; Shahidi and Ambigaipalan, 2018; Saini et al., 2021). Therefore, the target ω-3 LC-PUFAs and fatty acid intermediates produced by the GMOs are normally present in the human diet and/or synthesised in humans, and are not expected to be toxic.

42. ω-3 LC-PUFAs are highly susceptible to oxidation, with their oxidative stability inversely related to the number of carbon double bonds. Their primary oxidation products are lipid peroxides and lipid free radicals, which further decompose into a mix of secondary oxidation products including aldehydes and ketones. The rate of oxidation depends on temperature, exposure to light and exposure to oxygen (Arab-Tehrany et al., 2012; Albert et al., 2013; Miyashita, 2019). Even very low levels of oxidation produce volatile secondary oxidation products with offensive (rancid) odours and tastes. Flavour deterioration due to oxidation is a common problem in commercial fish oil products, despite use of antioxidant additives (Arab-Tehrany et al., 2012; Miyashita, 2019). In animal studies, highly oxidised PUFAs are reported to have toxic effects (Albert et al., 2013; Albert et al., 2016). For instance, pregnant rats fed fish oil where approximately 10% of the ω-3 LC-PUFAs were oxidized had much greater newborn mortality than control rats fed unoxidized fish oil or water (Albert et al., 2016).

43. A study of a GM canola line containing all of the fatty acid biosynthesis genes listed in Table 2 (Petrie et al., 2020) tested the stability of the seed oil profile when the seeds were stored at 24°C or 32°C for six months after harvest. There was no measured difference between the DHA levels of freshly harvested seeds and these stored seeds, although it is noted that the error bars of the measurements were up to ±4%, so it is possible that a small proportion of the DHA was oxidised during storage.

4.4 Characterisation of the GMOs

44. The introduced genes are not known to confer any phenotypic changes other than altered seed oil profile and herbicide tolerance. The applicant states that no unexpected phenotype has been observed while growing the GMOs in glasshouses.

45. A study of a GM canola line containing all of the introduced genes included in the current application (Petrie et al., 2020) evaluated agronomic parameters in the field. The GM canola line had a small reduction in total seed oil content compared to the non-GM parent canola cultivar, but there were no significant changes to yield, crop emergence, time to flowering and maturity, pod shattering, disease incidence or pest predation.

46. Gene constructs were introduced into the GMOs using *Agrobacterium*–mediated transformation. The GM lines will be propagated by seed to at least the third generation from the transformation within glasshouses before the field trial. *Agrobacterium* is not normally transmitted from one generation to the next via seed, so is not expected to be present in the GMOs proposed for release.

Section 5 The receiving environment

47. 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 GMOs; and background presence of the gene(s) used in the genetic modification (OGTR, 2013a).

48. Detailed information about the commercial cultivation and distribution of canola and Indian mustard in Australia is presented in the document *The Biology of Brassica napus L. (canola) and Brassica juncea (L.) Czern. & Coss. (Indian mustard)* (OGTR, 2017).

5.1 Relevant abiotic factors

49. The proposed release would occur in a range of geographic and climatic regions in NSW, Victoria, and Queensland. The most important abiotic factor limiting the geographical distribution of commercial canola and Indian mustard cultivation in Australia is water availability. Typically, canola can be grown in areas with annual rainfall over 325 mm and Indian mustard needs over 300 mm, although water requirements increase in hotter climates. Other abiotic stresses that can reduce canola or Indian mustard yield include soil acidity, waterlogging, frost and heat stress (GRDC, 2015, 2017).

5.2 Relevant biotic factors

50. The most important disease affecting canola and Indian mustard in Australia is blackleg, caused by the fungal pathogen *Leptosphaeria maculans.* Blackleg is most problematic in higher rainfall regions where a lot of canola is grown. Canola and Indian mustard can also be seriously damaged by stem rot caused by the fungus *Sclerotinia sclerotiorum* in wet springs. A range of other fungal or viral diseases sometimes reduce crop yield (McCaffery et al., 2009; GRDC, 2015, 2017).

51. Pests of canola and Indian mustard in eastern Australia include earth mites, aphids, moths, and Rutherglen bugs (McCaffery et al., 2009; GRDC, 2015, 2017).

52. Canola is highly susceptible to weed competition during the early stages of growth (GRDC 2015). Indian mustard and hybrid canola have greater seedling vigour than open-pollinated canola and so are more competitive with weeds (McCaffery et al., 2009; GRDC, 2015, 2017). Common weeds of Australian canola crops include grassy weeds, volunteer cereals, and weeds from the *Brassicaceae* family including wild radish (*Raphanus raphanistrum*), Indian hedge mustard (*Sisymbrium orientale*), shepherds purse (*Capsella bursa-pastoris*), wild turnip (*Brassica tournefortii*), charlock (*Sinapis arvensis*), turnip weed (*Rapistrum rugosum*) and Buchan weed (*Hirschfeldia incana*) (GRDC, 2015, 2017). To facilitate weed management, most canola varieties available in NSW and Victoria have a herbicide tolerance trait: imidizolinone tolerance, triazine tolerance, glyphosate tolerance (a GM trait) or dual-herbicide tolerance (Brown, 2021; Matthews et al., 2021).

5.3 Relevant agricultural practices

53. The applicant proposes that crop management practices for the GMOs would be the same as for commercial canola and Indian mustard crops, except for the proposed controls to restrict spread and

persistence of the GMOs (see Section 2.2). Standard cultivation practices for canola and Indian mustard in eastern Australia are discussed elsewhere (GRDC, 2015, 2017).

54. The applicant specifies that small areas/rows would be hand-planted or planted with a small plot seeder, while larger areas would be planted with commercial equipment. There may be multiple plots within a single planting area that have different planting and harvesting dates. Harvesting may occur by hand or with commercial equipment. Herbicides (including glufosinate), pesticides and drip/pipe irrigation may be used as necessary to manage the health of the GM crop.

5.4 Presence of related plants in the receiving environment

55. Canola and Indian mustard are primarily self-pollinating, but approximately 30% of seeds are produced by cross-pollination. Cross-pollination can be mediated by insects, wind or physical contact (OGTR, 2017).

56. Canola or Indian mustard have been reported to outcross in the field with the following species: *Brassica carinata*, *B. napus*, *B. juncea*, *B. oleracea, B. rapa*, *Hirschfeldia incana*, *Raphanus raphanistrum* and *Sinapis arvensis* (Ford et al., 2006; Warwick et al., 2009; Warwick and Martin, 2013). The applicant has indicated that they have commenced growing *B.carinata* in Australia as part of their biofuel development program (ASX Release 1 Feb 2022).

57. Canola (*B. napus*) is widely grown as an oilseed crop in NSW and Victoria, but rarely grown in Queensland (ABARES, 2021). The proposed trial sites in NSW and Victoria, but not Queensland, are likely to be located in commercial canola growing regions. Indian mustard (*B. juncea*) is a minor oilseed crop grown in similar areas to canola. Cabbage (*B. oleracea*) and turnip (*B. rapa*) are cultivated as horticultural crops. These four species are also naturalised in parts of NSW, Victoria and Queensland (VICFLORA, accessed 27 Jan 2022).

58. Buchan weed (*H. incana*), wild radish (*R. raphanistrum*) and charlock (*S. arvensis*) are widespread weeds in NSW, Victoria and south-east Queensland (New South Wales Flora Online, accessed 27 Jan 2022; Weeds Australia, accessed 27 Jan 2022). As discussed in Section 5.2, these species are common weeds in canola crops.

5.5 Presence of similar genes and their products in the environment

59. Five of the introduced genes involved in fatty acid biosynthesis are sourced from microalgae that are present in the marine environment (*Pavlova salina*, *Micromonas pusilla* and *Pyramimonas cordata)*. People may naturally encounter the genes and encoded proteins through contact with sea water or seafood. In addition, humans and other mammals have similar, endogenous genes encoding enzymes responsible for converting dietary ALA into ω-3 LC PUFAs (Zarate et al., 2017; Shahidi and Ambigaipalan, 2018; Saini et al., 2021).

60. Two of the introduced genes involved in fatty acid biosynthesis are sourced from the yeasts *Pichia pastoris* and *Lachancea kluyveri*. These yeasts were isolated from trees and soil in the Northern Hemisphere. Both of these yeasts are present in the New Zealand environment (Zhang et al., 2010), likely due to import of host tree species from Europe, and are also expected to be present in Australia.

61. The *pat* gene was obtained from the common soil bacterium *Streptomyces viridochromogenes.* The *pat* gene or the similar *bar* gene from *S. hygroscopicus* are also present in many types of GM canola or cotton authorised for commercial release in Australia (licences DIR 021/2002, DIR 062/2005, DIR 091, DIR 108, DIR 138, DIR 143, DIR 145, DIR 155, DIR 173, DIR 175 and DIR 178).

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

Approvals by the Regulator

62. GM canola lines containing all introduced genes proposed for release under the current application were previously approved for field trials under licences DIR 123 and DIR 163 and for commercial release under licence DIR 155. The GM canola line approved for commercial release under licence DIR 155 is known as DHA canola (NS-B50027-4).

63. GM Indian mustard lines containing all introduced genes proposed for release under the current application were previously approved for field trials under licence DIR 149.

64. There are no reported adverse effects from these previous releases.

Approvals by other government agencies

65. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has assessed the safety of food derived from DHA canola and approved its food products for commercial sale (FSANZ, 2017).

6.2 International approvals

66. DHA canola was deregulated for commercial cultivation in the United States in 2018. DHA canola was approved for food, feed, and commercial cultivation in Canada in 2020.

Chapter 2 Risk assessment

Section 1 Introduction

67. 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). Risks are identified within the established risk context (Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

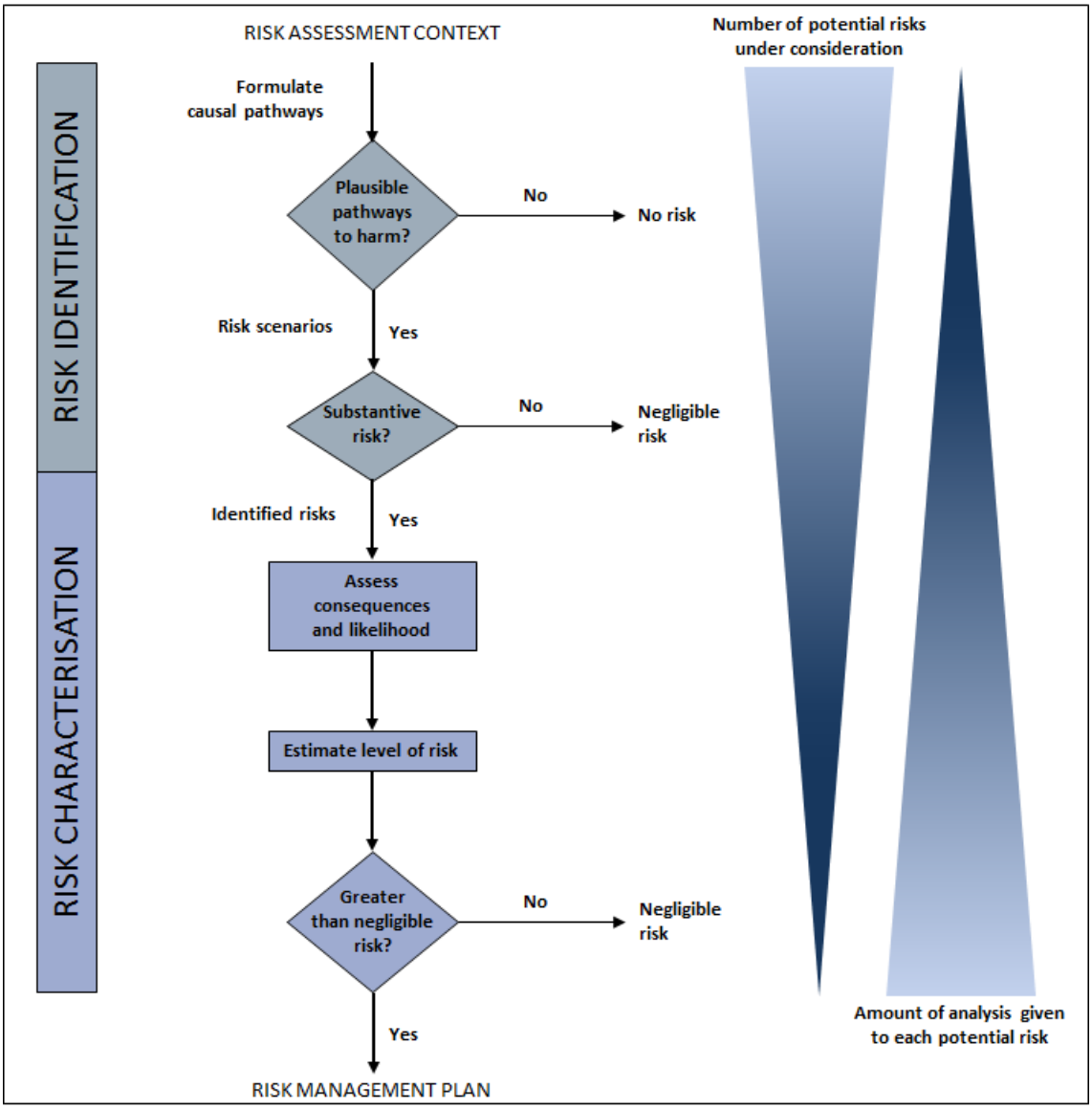

Figure 4. The risk assessment process

68. The Regulator uses a number of techniques to identify risks, 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, as this approach addresses the full range of potential adverse outcomes associated with 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 examined in RARMPs prepared for licence applications for the same or similar GMOs are also considered.

69. Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating

plausible causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are risk scenarios. These risk scenarios are screened to identify those that are considered to have a reasonable chance of causing harm in the short or long term. Pathways that do not lead to harm, or those that could not plausibly occur, do not advance in the risk assessment process (Figure 4), i.e., the risk is considered to be no greater than negligible.

70. Risks identified as being potentially greater than negligible are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). Risk evaluation then combines the Consequence and Likelihood assessments to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 Risk identification

71. Postulated risk scenarios have three components (Figure 5):

i. the source of potential harm (risk source)

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

iii. potential harm to people or the environment.

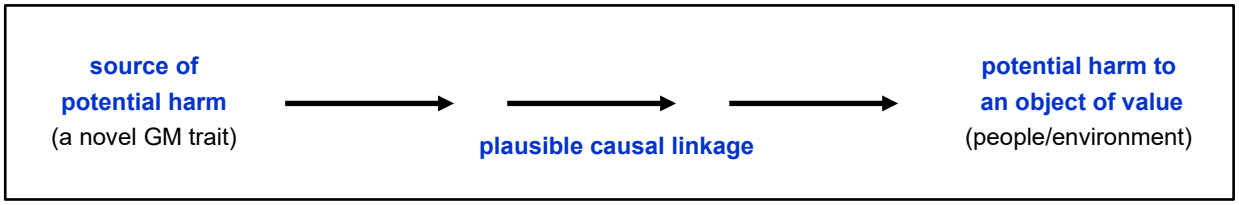

Figure 5. Risk scenario

72. When postulating relevant risk scenarios, the risk context is taken into account, including the following factors detailed in Chapter 1:

- the proposed dealings
- the proposed limits including the extent and scale of the proposed dealings
- the proposed controls to restrict the spread and persistence of the GMOs and
- the characteristics of the parent organism(s).

2.1 Risk source

73. 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.

74. As discussed in Chapter 1, the GM canola and Indian mustard lines have been modified by the introduction of up to seven genes involved in fatty acid biosynthesis. These introduced genes will be considered further as a source of potential harm.

75. The GM canola and Indian mustard lines may also contain the introduced *pat* gene which confers tolerance to glufosinate herbicide and was used as a selectable marker. The applicant also proposes to use glufosinate herbicide as a crop management tool under this licence. As discussed in Chapter 1, the *pat* gene and its products, as well as the toxicity of glufosinate metabolites have been considered in multiple previous RARMPs and found to pose negligible risks. In addition, the APVMA has regulatory responsibility for agricultural chemicals, including herbicides, in Australia. For these reasons, the *pat* gene will not be further considered as a source of potential harm.

76. The introduced genes are controlled by regulatory sequences. These were originally derived from plants, plant viruses and a bacterium (Table 3). Regulatory sequences are naturally present in all plants, and the introduced elements are expected to operate in similar ways to endogenous elements. These sequences are DNA that is not expressed as a protein, so exposure is to the DNA only and dietary DNA has

no toxicity (Society of Toxicology, 2003). Hence, potential harms from the regulatory sequences will not be further assessed for this application.

77. Genetic modifications involving introduction of genes have the potential to cause unintended effects in several ways. These include insertional effects such as interruptions, deletions, duplications or rearrangements of the genome, which can lead to altered expression of endogenous genes. There could also be increased metabolic burden due to expression of the introduced proteins, 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, and there are no documented cases where conventional breeding has resulted in the production of a novel toxin or allergen in a crop (Steiner et al., 2013). Therefore, the potential for the processes of genetic modification to result in unintended effects will not be considered further.

2.2 Causal pathway

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

- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the environment at the site(s) of release
- agronomic management practices for the GMOs
- spread and persistence of the 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)
- unauthorised activities.

79. Although all of these factors are taken into account, some are not included in risk scenarios because they have been considered in previous RARMPs.

80. The potential for horizontal gene transfer (HGT) from GMOs to species that are not sexually compatible, and any possible adverse outcomes, have been reviewed in the literature (Keese 2008) and assessed in many 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, no substantive risk was identified in previous assessments and HGT will not be further considered for this application.

81. The potential for increased fitness of pest species due to the consumption of ω-3 LC-PUFAs has been previously considered in the RARMP for commercial canola licence DIR 155. No substantive risk was identified in this previous assessment for commercial release and there has been no further evidence to the contrary. Therefore, increased fitness of pest species will not be further considered for this application.

82. The potential for unauthorised activities to lead to an adverse outcome has been considered in many previous RARMPs, most recently in the RARMP for DIR-117 (OGTR, 2013b). In previous assessments of unauthorised activities, no substantive risk was identified. The Act provides for substantial penalties for unauthorised dealings with GMOs or non-compliance with licence conditions, and also requires the Regulator to have regard to the suitability of an applicant to hold a licence prior to the issuing of the

licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, unauthorised activities will not be considered further.

2.3 Potential harm

83. Potential harms from GM plants are based on those used to assess risk from weeds (Virtue, 2008; Keese et al., 2014) including:

- 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).

84. 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

85. Three risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 4 and examined in detail in Sections 2.4.1 – 2.4.3.

86. In the context of the activities proposed by the applicant and considering both the short and long term, none of the risk scenarios gave rise to any substantive risks.

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

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced genes for altered oil content	Cultivation of GM canola and Indian mustard at trial sites ⬇ Exposure of people and other desirable organisms to products of the introduced genes	Increased toxicity or allergenicity for people OR increased toxicity to other desirable organisms	No	• The GM canola and Indian mustard would not be used as commercial human food or animal feed • The limits and controls of the field trial would restrict exposure of people and other desirable organisms to the GM plants • The proteins encoded by the introduced genes are not expected to be toxic or allergenic • Oil containing ω-3 LC-PUFAs is not expected to be toxic • Oxidation products of ω-3 LC-PUFAs could be toxic, but people will not consume these products, and animals are highly unlikely to consume toxic levels due to low oxidation rates over the expected lifetime of seeds.
2	Introduced genes for altered oil content	Cultivation of GM canola and Indian mustard 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 people OR increased toxicity to other desirable organisms OR reduced establishment or yield of desirable plants	No	• The controls of the field trial would minimise dispersal or persistence of GM seeds • GM canola and Indian mustard are susceptible to standard weed management measures • As discussed in Risk Scenario 1, the genetic modifications are not expected to cause increased toxicity or allergenicity • Canola and Indian mustard have limited ability to compete with other plants and the genetic modifications are not expected to increase their competitiveness.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
3	Introduced genes for altered oil content	Cultivation of GM canola and Indian mustard at trial sites ⬇ Pollen from GM plants dispersed outside the trial sites ⬇ Outcrossing with sexually compatible plants ⬇ Establishment of populations of hybrid GM plants expressing the introduced genes in the environment	Increased toxicity or allergenicity for people OR increased toxicity to other desirable organisms OR reduced establishment or yield of desirable plants	No	• The controls of the field trial would minimise pollen flow to sexually compatible plants outside the trial sites • As discussed in Risk Scenario 1, the genetic modifications are not expected to cause increased toxicity or allergenicity • As discussed in Risk Scenario 2, the genetic modifications are not expected to increase ability to compete with other plants.

2.4.1 Risk Scenario 1

Risk source	Introduced genes for altered oil content
Causal pathway	⬇ Cultivation of GM canola and Indian mustard at trial sites ⬇ Exposure of people and other desirable organisms to products of the introduced genes ⬇
Potential harm	Increased toxicity or allergenicity for people OR Increased toxicity to other desirable organisms

Risk source

87. The source of potential harm for this postulated risk scenario is the introduced genes for altered oil content in GM canola and Indian mustard plants.

Causal pathway

88. The GM canola and Indian mustard would be grown at the trial sites. As the introduced genes for altered oil content are controlled by seed-specific promoters, the encoded proteins would be produced in the GM seeds. The seed oil is expected to be enriched in target ω-3 LC-PUFAs and may also be enriched in fatty acids that are intermediates in the biosynthesis pathway of the target ω-3 LC-PUFAs. People and other desirable organisms could be exposed to GM seeds containing the introduced proteins and seed oil enriched in ω-3 LC-PUFAs.

89. The GM canola and Indian mustard would not be used for commercial human food. Only authorised and trained trial staff would be permitted to deal with the GM plants and their seeds. Therefore, there is little potential for the public to be exposed to GM seeds grown at the trial sites.

90. Trial staff would handle the GM seeds and plant material produced by processing of the GM seeds. Workers could be exposed to the introduced proteins and seed oil enriched in ω-3 LC-PUFAs by dermal contact and inhalation.

91. The applicant proposes human sensory testing of GM plant material to assess the feel, smell, taste and appearance of the seed oil or food products containing the oil. These tests would involve negligible consumption of the oil as the products are not intended to be swallowed during testing. Canola oil is highly

refined and does not contain detectable amounts of protein and DNA. People participating in sensory testing could be exposed to seed oil enriched in ω-3 LC-PUFAs by dermal contact, contact with mucous membranes and inhalation.

92. The GM canola and Indian mustard would not be used for commercial animal feed and livestock would not be permitted to graze the trial sites. Therefore, livestock are not expected to be exposed to GM seeds grown at the trial sites.

93. Desirable organisms, such as native mammals, birds, and insects could enter the trial sites and consume GM seeds. Soil organisms, such as earthworms, might come into contact with decomposing GM canola seed. The limited size and duration of the field trial would restrict the number of desirable organisms exposed to GM seeds grown at the trial sites. Pollinators such as honeybees would have minimal or no exposure to the products of the introduced genes as expression is limited to the seed rather than nectar or pollen.

94. The applicant proposes animal feeding trials with GM plant material, which may include trials with rodents, chickens and farmed fish species. The experimental animals would be exposed to the introduced proteins and/or seed oil enriched in ω-3 LC-PUFAs.

Potential harm

95. As discussed in Chapter 1, Section 4.2, none of the introduced proteins are expected to be toxic or allergenic.

96. As discussed in Chapter 1, Section 4.3, the target ω-3 LC-PUFAs and fatty acid intermediates are not expected to be toxic. One laboratory study reported that adult individuals of the pest cabbage white butterfly (*Pieris rapae*) were heavier and had an increased rate of wing deformities when fed on diets containing additional ω-3 LC-PUFAs (Hixson et al., 2016). However, white butterfly caterpillars feed on leaves, and the altered oils in the GM canola and Indian mustard reviewed under this application are confined to the seeds. In addition, no information was provided in the study about possible oxidation products (see next paragraph) that could influence toxicity.

97. As discussed in Chapter 1, Section 4.3, ω-3 LC-PUFAs are highly susceptible to oxidation, and the oxidation products can have toxic effects if consumed. There is little published information regarding the level of toxicity of ω-3 LC-PUFA oxidation products. When pregnant rats were fed heavily oxidised fish oil as a large component of their diet for the entire period of pregnancy, this caused increased newborn mortality but did not increase mortality of the mothers (Albert et al., 2016). Therefore, if GM seed oil were heavily oxidised, this could increase mortality of neonate animals whose mothers consumed the GM seed. It is uncertain whether toxic effects could occur in other animals feeding on the GM seed, especially animals that may be particularly sensitive to the oxidation products.

98. A study of a GM canola line containing all of the introduced genes for altered oil content tested the stability of seed oil during six months of seed storage after harvest and did not detect any changes in the levels of ω-3 LC-PUFAs (Petrie et al., 2020). This suggests that oxidation of ω-3 LC-PUFAs in seeds occurs at a very slow rate under storage conditions, so GM seeds stored for planting or experimentation would not contain heavily oxidised oil. Oxidation may occur more rapidly in seeds that are lost during harvest and remain on the soil surface, as the oxidation rate of ω-3 LC-PUFAs is increased by exposure to air and light (Arab-Tehrany et al., 2012; Albert et al., 2013; Miyashita, 2019). However, seeds on the soil surface are very susceptible to predation and would probably be consumed before much oxidation could occur. For example, in a Canadian study where canola seeds were left on the soil surface, 42–77% of seeds were consumed by invertebrate seed predators within a week (Kulkarni et al., 2017). Therefore, desirable organisms are highly unlikely to be exposed to toxic levels of ω-3 LC-PUFA oxidation products through consumption of GM seeds. The timeframes for oxidation of ω-3 LC-PUFAs in seeds under different conditions are an area of uncertainty for this risk assessment.

99. As discussed in Chapter 1, Section 6.1, GM canola and Indian mustard lines containing all of the introduced genes for altered oil content have been previously approved by the Regulator for field trials and commercial release. To date, no adverse effects have been reported from these releases.

Conclusion

100. Risk scenario 1 is not identified as a substantive risk because the limits and controls of the field trial would restrict exposure of people and other desirable organisms to the GM plants, the introduced proteins are not expected to be toxic or allergenic, and oil containing ω-3 LC-PUFAs is not expected to be toxic. Although oxidation products of ω-3 LC PUFAs could be toxic, people will not consume these products and animals are highly unlikely to consume toxic levels. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk Scenario 2

Risk source	Introduced genes for altered oil content
Causal pathway	⬇ Cultivation of GM canola and Indian mustard 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 people OR Increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable plants

Risk source

101. The source of potential harm for this postulated risk scenario is the introduced genes for altered oil content in GM canola and Indian mustard plants.

Causal pathway

102. The GM canola and Indian mustard would be grown at the trial sites. GM seeds could be physically dispersed outside the trial sites by human activity, animal activity, wind, or water. GM seeds could also persist on trial sites after completion of the trial. These GM seeds could grow in the environment and establish populations of volunteer GM plants.

103. Viable GM canola and Indian mustard seeds could be dispersed outside the trial sites by human activity, such as transport of seeds and movement of agricultural machinery. To minimise dispersal of GM seeds by human activity, the applicant proposes to clean all equipment used with the GM plants after use, and to transport all GM seed in accordance with the Regulator's guidelines for the transport of GMOs.

104. GM seeds could be dispersed outside the trial sites by animal activity. Canola and Indian mustard seeds have no specific adaptions, such as burrs or hooks, for dispersal by animals (OGTR, 2017). Dispersal of viable canola seed via endozoochory (consumption and excretion of seed) by birds only occurs at very low levels, with less than 0.1% of seeds surviving after ingestion (Twigg et al., 2008; Woodgate et al., 2011). Canola and Indian mustard seeds could be transported short distances by hoarding animals, such as ants and mice. As outlined in Risk Scenario 1, the limited size and duration of the field trial would restrict the number of desirable organisms exposed to GM seeds grown at the trial sites. The applicant proposes that monitoring zones around trial sites would be inspected for volunteers.

105. Canola and Indian mustard seeds lack specialised structures that would assist their dispersal by wind (OGTR, 2017). However, the GM canola may be windrowed prior to harvesting, and under strong wind conditions plant material could disperse outside trial sites. The applicant proposes that monitoring zones around trial sites would be inspected for volunteers.

106. GM canola and Indian mustard seeds could be dispersed by water during flooding or heavy runoff, although seeds are unlikely to remain viable after prolonged exposure to water (OGTR, 2017). The applicant

proposes to locate the trial sites at least 50 m from waterways to minimise the potential for seed dispersal during flooding.

107. During harvest of the GM canola and Indian mustard, a small percentage of the GM seeds are expected to be lost and to remain on the trial sites. Viable canola and Indian mustard seeds can persist in the seedbank for several years (OGTR, 2017). It is unlikely that the genetic modifications for altered seed oil composition would affect seed persistence. A field study of seedbank persistence in a GM canola line with altered oil content found no difference between seedbank persistence of the GM line and the non-GM control at the completion of the trial, which was 14-19 months after seed burial (Linder and Schmitt, 1995). To minimise persistence of residual GM seeds on the trial sites, the applicant proposes to promote seed germination by light post-harvest tillage and irrigation. During a post-harvest monitoring period, the applicant would regularly inspect the trial sites and destroy any GM volunteers, until volunteers cease to emerge.

108. The suitability of the proposed controls to manage GM seed dispersal and persistence is discussed in detail in Chapter 3, Section 3.1.

109. If GM canola and Indian mustard seeds were dispersed outside trial limits, it is unlikely that they would establish ongoing volunteer populations. Even in environments without active weed management, volunteer canola populations along transportation routes rely on recurrent spillages to persist (Yoshimura et al., 2006) and volunteer canola dispersed into natural areas was reported to become extinct within 3 seasons (Busi and Powles, 2016). The genetic modifications for altered seed oil composition are not expected to affect the ability of volunteers to survive in the environment. A GM canola line containing the introduced genes had no changes in agronomic traits compared to the non-GM control (Petrie et al., 2020).

110. In agricultural areas of Australia where canola and Indian mustard are grown, volunteer populations are controlled by weed management measures. Effective methods for control of canola volunteers include grazing, mowing, cultivation and application of a range of knockdown or selective herbicides (AOF, 2019). The genetic modifications for altered seed oil composition would not affect the susceptibility of GM volunteers to standard weed management measures.

Potential harm

111. As discussed in Risk Scenario 1, it is not expected that the GM canola and Indian mustard would have increased toxicity or allergenicity for people or increased toxicity to other desirable organisms.

112. Populations of volunteer GM canola and Indian mustard could reduce establishment or yield of desirable plants. GM volunteers could directly compete with agricultural crops, pastures or native vegetation. GM volunteers could also reduce yield of commercial canola and Indian mustard crops by providing a reservoir for pathogens, such as the important fungal diseases blackleg and stem rot (see Chapter 1, Section 5.2).

113. Canola is considered a less competitive crop species than wheat or barley (GRDC, 2011), which are the main crops grown in eastern Australia (ABARES, 2021). Indian mustard has a similar phenotype to canola, although it may be slightly more competitive due to greater seedling vigour (GRDC, 2015, 2017). All domesticated crop plant species are expected to be poor competitors with pasture species or established native vegetation. Therefore, canola and Indian mustard volunteers have limited ability to compete with desirable plants. The genetic modifications for altered seed oil composition are not expected to increase the competitiveness of GM plants. The biological purpose of plant seed oil is to provide an energy source for germination and seedling establishment, and highly unsaturated oil provides less energy per carbon atom than saturated or monounsaturated oil (Sanyal and Decocq, 2016). It is therefore highly unlikely that the GM canola and Indian mustard seeds will have any significant advantage over parental seeds in plant establishment.

114. Blackleg and stem rot diseases affect vegetative parts of canola and Indian mustard plants rather than seeds (GRDC, 2015, 2017). The genetic modifications for altered seed oil are not expected to increase the ability of GM plants to act as reservoirs for these pathogens.

Conclusion

115. Risk Scenario 2 is not identified as a substantive risk because the controls of the field trial would minimise dispersal or persistence of GM seeds, GM canola and Indian mustard are susceptible to standard weed management measures, the genetic modifications are not expected to increase toxicity or allergenicity, and the genetic modifications are not expected to increase competitiveness with other plants. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk Scenario 3

Risk source	Introduced genes for altered oil content
Causal pathway	⬇ Cultivation of GM canola and Indian mustard at trial sites ⬇ Pollen from GM plants dispersed outside the trial sites ⬇ Outcrossing with sexually compatible plants ⬇ Establishment of populations of hybrid GM plants expressing the introduced genes in the environment ⬇
Potential harm	Increased toxicity or allergenicity for people OR Increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable plants

Risk source

116. The source of potential harm for this postulated risk scenario is the introduced genes for altered oil content in GM canola and Indian mustard plants.

Causal pathway

117. The GM canola and Indian mustard would be grown at the trial sites. Pollen from the GM plants could be transported out of the trial sites by wind or insect vectors and fertilise sexually compatible plants. Hybrid seeds containing the introduced genes could be harvested by farmers and planted as a crop or could grow as volunteers.

118. 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.

119. Canola and Indian mustard are primarily self-pollinating, but approximately 30% of seeds are produced by cross pollination. Outcrossing decreases rapidly with distance, with the majority of cross-pollination occurring over distances less than 10 m (OGTR, 2017). The introduced genes for altered oil content are only expressed in seeds and are not expected to affect the pollen dispersal characteristics of the GM canola and Indian mustard.

120. The GM canola and Indian mustard could outcross with nearby canola or Indian mustard crops or volunteers, if there is synchronicity of flowering. As discussed in Chapter 1, Section 5.4, canola or Indian mustard can also occasionally hybridise with the related horticultural crops *B. oleracea*, *B. rapa,* and *B. carinata* and the related weeds *H. incana*, *R. raphanistrum,* and *S. arvensis.*

121. The applicant has proposed control measures to minimise pollen flow from GM plants growing on the trial sites to sexually compatible plants outside the trial sites (Chapter 1, Section 2.2). During flowering of the GM plants, each planting area would be (a) covered by an insect proof tent and surrounded by a monitoring zone and isolation zone, or (b) surrounded by a pollen trap, monitoring zone and isolation zone, or (c) surrounded by a monitoring zone and a large isolation zone. In addition, any GM volunteers growing on the trial sites after harvest would be destroyed prior to flowering.

122. The suitability of the proposed controls to manage pollen flow is discussed in detail in Chapter 3, Section 3.1.

123. If pollen from GM plants were to fertilise plants in a nearby commercial canola or Indian mustard crop, the farmer could harvest some of the crop either for commercial human food or animal feed, or as planting seed. However, even in the complete absence of measures to restrict pollen flow, outcrossing rates between neighbouring commercial canola fields are less than 0.1% under Australian conditions (Rieger et al., 2002). Due to the proposed limits and controls, it would be expected that the outcrossing rates between the GM canola and any neighbouring commercial canola would be even lower. Therefore, the seed described in this risk pathway could only contain a very low proportion of hybrid GM seed, and it would be mixed with large volumes of commercial crops grown distant from the proposed trial sites, so people and other desirable organisms could only be exposed to very low levels of the hybrid GMOs.

124. If pollen from GM plants fertilised sexually compatible plants growing as crops, volunteers or weeds, the hybrid GM seeds could grow as volunteers. Populations of hybrid GM volunteers could be consumed by other desirable organisms or could reduce the establishment or yield of desirable plants.

Potential harm

125. As discussed in Risk Scenario 1, the GM canola and Indian mustard are not expected to have greater toxicity or allergenicity for people or greater toxicity to other desirable organisms than non-GM canola or Indian mustard. Similarly, in hybrids between the GM plants and sexually compatible plants, the genetic modifications would not increase toxicity or allergenicity.

126. As discussed in Risk Scenario 2, the GM canola and Indian mustard are not expected to have greater competitiveness than non-GM canola or Indian mustard. Similarly, in hybrids between the GM plants and sexually compatible plants, the genetic modifications would not increase ability to compete with other plants.

Conclusion

127. Risk Scenario 3 is not identified as a substantive risk because the controls of the field trial would minimise pollen flow to sexually compatible plants outside the trial sites, the genetic modifications are not expected to cause increased toxicity or allergenicity, and the genetic modifications are not expected to increase the ability to compete with other plants. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

128. Uncertainty is an intrinsic property of risk analysis and is present in all aspects of risk analysis. This is discussed in detail in the Regulator's Risk Analysis Framework document.

129. 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.

130. 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.

131. For DIR 188, uncertainty is noted particularly in relation to the potential for toxicity of ω-3 LC-PUFA oxidation products to animals consuming the seeds of the GM canola and Indian mustard.

132. 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.

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

Section 4 Risk evaluation

134. 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.

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

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

136. Three 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 4 and include:

- the GM plants would not be used as commercial human food or animal feed
- limits on the size and duration of the proposed release
- controls proposed by the applicant to restrict the spread and persistence of the GM canola and Indian mustard plants and their genetic material (see Chapter 3 for discussion of their suitability)
- the products of the introduced genes are not expected to be toxic or allergenic
- GM canola and Indian mustard volunteers could be controlled by standard weed management measures.

137. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM canola and Indian mustard 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. The Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment[1].

[1] 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.

Chapter 3 Risk management plan

Section 1 Background

138. 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.

139. 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.

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

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

Section 2 Risk treatment measures for substantive risks

142. 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 and Indian mustard. These risk scenarios were considered in the context of the scale of the proposed release (Chapter 1, Section 2.1), the proposed control measures (Chapter 1, Section 2.2), and the receiving environment (Chapter 1, Section 5), 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

143. 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 proposed 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 detail in the licence.

3.1 Licence conditions to limit and control the release

144. Sections 2.1 and 2.2 of Chapter 1 provide details of the limits and controls proposed by Nuseed in their application. Many of these are discussed in the three risk scenarios considered for the proposed release in Chapter 2. The appropriateness of these limits and controls is considered further in the following sections.

3.1.1 Consideration of limits proposed by Nuseed

145. The applicant proposes that the field trial would take place at up to twenty sites per year. Ten of these sites would have a maximum planting area of 10 ha and ten sites would have a maximum planting area of 5 ha, so the total trial area would be up to 150 ha/year. Sites would be selected from 96 local government areas in NSW, Victoria and Queensland. The duration of the field trial would be five years, from November 2022 to January 2028. The limited size and duration of the trial would restrict the potential exposure of people and other desirable organisms to the GMOs (Risk Scenario 1).

146. The applicant proposes that only trained and authorised staff would be permitted to deal with the GMOs. These limits would restrict the number of people exposed to the GMOs (Risk Scenario 1).

3.1.2 Consideration of proposed controls regarding exposure to the GMOs

147. The applicant proposes that GM plant material would not be used in commercial human food or animal feed. The applicant proposes to use GM plant material in animal feeding trials and possibly human taste testing trials (as part of broader sensory testing). The licence requires that GM plant material must not be used as food for humans or feed for animals, except for use in specified animal feeding experiments or human sensory testing. Animal feeding experiments must be approved by an Animal Ethics Committee operating under the Australian Code for the Care and Use of Animals for Scientific Purposes and human sensory testing must be approved by a Human Research Ethics Committee in accordance with the National Statement on Ethical Conduct in Human Research. These conditions would restrict the exposure of people and other desirable organisms to the GMOs (Risk Scenario 1).

3.1.3 Consideration of proposed controls regarding pollen flow from the GMOs

148. The applicant proposed three different options to control pollen flow from the trial sites while the GMOs are flowering.

149. The first option to control pollen flow is to surround the planting area with a 50 m monitoring zone and a 1 km isolation zone. The GMOs would not be planted at a trial site if any plants that are sexually compatible with canola or Indian mustard were being grown in the monitoring or isolation zones. The monitoring zone would be inspected at least once every 35 days from 14 days prior to flowering of the GMOs until the GMOs are harvested, to ensure that it is free from any sexually compatible plants. The isolation zone would be inspected at least once every 35 days from 14 days prior to flowering of the GMOs until the GMOs complete flowering, to ensure that it is free from intentionally planted sexually compatible plants. This option was proposed for previous GM canola field trials and was considered an effective means of restricting pollen flow from canola (e.g. DIR 164 (OGTR, 2018b)).

150. The pattern of pollen movement for *B. juncea* is similar to *B. napus* (Salisbury, 2006). The Canadian Regulations and Procedures for Pedigreed Seed Crop Production (CSGA, 2022) require that foundation production of male sterile *B. juncea* or *B. napus* seed must be separated from other *B. juncea* or *B. napus* plants by an 800 m isolation distance, of which the first 50 m must be practically free from related plants, and the remaining distance must be reasonably free from related plants. Therefore, the proposed 50 m monitoring zone and 1 km isolation zone, which are more stringent than these Canadian requirements, are considered effective measures to restrict pollen flow from Indian mustard.

151. The second option to control pollen flow is to surround the planting area with a 15 m pollen trap of non-GM canola plants, a 50 m monitoring zone and a 400 m isolation zone. The pollen trap would be managed to flower at the same time as the GMOs. Pollen trap plants may provide sufficient forage for incoming pollinating insects that they do not visit the GM plants, and any insects that reach the GM plants are expected to deposit most GM pollen on pollen trap plants while exiting the trial site. Pollen trap plants may also absorb some pollen dispersed by wind. Therefore, the use of a pollen trap justifies reducing the isolation zone from 1 km to 400 m. As a non-GM pollen trap or buffer zone can also serve the same function as an unplanted monitoring zone (Hüsken and Dietz-Pfeilstetter, 2007), it is considered unnecessary to have both a pollen trap and a full-sized 50 m monitoring zone. The draft licence permits the applicant to use a 15 m pollen trap combined with a 35 m monitoring zone.

152. The third option to control pollen flow is to cover the planting area with an insect proof tent, and to surround the planting area with a 10 m monitoring zone and a 400 m isolation zone. The tents would be in place from at least seven days before flowering until the GMOs complete flowering, and would be inspected for damage fortnightly and after any extreme weather event. The tents are expected to prevent all insect-mediated pollen flow and to greatly reduce wind-mediated pollen flow. Therefore, the use of an insect-proof tent justifies a reduced monitoring zone and isolation zone. If there is a mixture of tented and non-tented plots within a planting area, either the first or second option is considered suitable for pollen flow management.

153. The proposed measures to control pollen flow would minimise outcrossing between the GMOs grown on the trial sites and sexually compatible plants growing outside the trial sites (Risk Scenario 3).

154. After harvest of the trial sites, the applicant proposes to monitor the sites for volunteers (see Section 3.1.5). The applicant would inspect at least once every 35 days, in order to find and destroy volunteers before they flower. These post-harvest inspections were proposed for previous GM canola field trials and were considered an effective means of restricting pollen flow from GM canola volunteers to plants outside the trial sites (e.g. DIR 164 (OGTR, 2018b)).

155. However, studies from Australia and North America have reported that canola-quality *B. juncea* varieties often begin flowering earlier than comparable canola varieties (Gunasekera et al., 2006; Gan et al., 2007; Riar, 2015; Hunter et al., 2017). Canola-quality *B. juncea* varieties planted in spring were reported to begin flowering 44-46 days after sowing (Gan et al., 2007; Hunter et al., 2017). This suggests that GM Indian mustard volunteers germinating in spring or summer could flower around 35-40 days after emergence. Therefore, the draft licence requires post-harvest inspections at least once every 30 days for any trial sites that grew Indian mustard and at least once every 35 days for any trial sites that grew only canola. These post-harvest monitoring requirements would minimise outcrossing between GM volunteers and sexually compatible plants growing outside the trial sites (Risk Scenario 3).

3.1.4 Consideration of proposed controls regarding dispersal of the GMOs

156. The applicant proposes to treat any non-GM canola and Indian mustard plants grown in planting areas or pollen traps like the GMOs. These non-GM plants may be mingled with or fertilised by the GM plants and it is therefore necessary to handle the non-GM plants in the same way as the GMOs to manage the dispersal or persistence of GM seed.

157. The applicant proposes that the GM canola and Indian mustard would be harvested separately from other crops, to avoid inadvertent seed mixing. Any equipment used with the GMOs would be cleaned as soon as practicable and before use for any other purpose, to avoid movement of viable plant material together with equipment. The applicant would contain the GM seeds during transport and storage in accordance with the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs. These measures would minimise human-mediated dispersal of GM seeds (Risk Scenario 2)

158. The applicant proposes to locate trial sites at least 50 m away from waterways. The licence requires that planting areas and pollen traps) must be at least 50 m from waterways and must not be located in flood-prone areas, and that any extreme weather events must be reported to the Regulator. These measures would minimise dispersal of GM seeds by flooding (Risk Scenario 2).

159. GM canola and Indian mustard seeds could be dispersed short distances from the trial sites during sowing, windrowing or harvest activities, by pod shattering, by seed-hoarding behaviours of animals such as ants or rodents, or by strong winds or runoff after heavy rain. As described in Section 3.1.3, the planting areas would be surrounded by monitoring zones that are inspected while the GMOs are growing, so any volunteers growing from dispersed GM seeds during this period would be detected and destroyed. The applicant also proposes to inspect the monitoring zones after harvest to destroy any volunteers growing from dispersed GM seeds. The size of the monitoring zones is 10 m, 35 m or 50 m, depending on the measures used to control pollen flow. As the short-distance seed dispersal mechanisms listed above are unlikely to transport seeds further than 10 m from the trial sites, the licence only requires post-harvest inspections of the innermost 10 m of each monitoring zone.

160. The licence includes additional conditions to manage short-distance dispersal of GM seeds. These include taking measures to minimise dispersal of windrowed GMOs by wind or rain, requiring the trial site to be cleaned within 14 days after harvest by a method that removes GM seeds from the soil surface, and requiring post-harvest inspections of any area used to clean equipment or any other area where GMOs are known to have dispersed. This combination of controls would minimise short-distance dispersal of GM seeds leading to establishment of volunteer populations outside the trial sites (Risk Scenario 2).

3.1.5 Consideration of proposed controls regarding persistence of the GMOs

161. After harvest of each trial site, the applicant proposes to destroy GMOs not required for further evaluation or future trials. This would involve both cleaning the trial site within 14 days after harvest in a manner that destroys any surviving GM plants, and destroying any harvested GM seed that is not required for experimentation or future planting. The applicant's proposed methods for destruction of GMOs were approved for previous canola field trials (e.g. DIR 164 (OGTR, 2018b)). In addition, uprooting of plants and crushing or grinding of seeds are considered to be effective methods of destruction and have been included as options in the licence.

162. To deal with the case of failed crops that are not harvested, licence conditions require that all GMOs planted in a planting area must be harvested or destroyed within nine months after planting of the first GMOs in that planting area. This condition will also limit the spread and persistence of GMOs if there are multiple harvest dates within a single plot. In addition, if all GMOs in a planting area have been destroyed, then the area is considered to have been harvested and cleaned. GMOs are defined as GM plants and any viable seed, and the term 'destruction' includes both, as appropriate for the stage of the GM plants at the time of destruction.

163. The applicant proposes to monitor trial sites after harvest and destroy any volunteers that emerge. The areas that would be monitored are the planting area, the pollen trap, and other areas where GM seed may have dispersed, as discussed in Section 3.1.4. The frequency of inspections of the trial sites are discussed in Section 3.1.3. The proposed duration of monitoring is at least 24 months, and until the site is free of volunteer canola or Indian mustard plants for at least 12 months. This monitoring duration was proposed for previous GM canola field trials and was considered effective for managing persistence of canola seed (e.g. DIR 164 (OGTR, 2018b)).

164. In minimum-tillage Australian farms, the canola seedbank is reported to decline rapidly, and no viable seed was recovered from the seedbank by 2.5 years after canola harvest (Baker and Preston, 2008). Similarly, OGTR monitoring data for nine GM canola trial sites planted in 2015 found that in most sites no canola volunteers emerged more than 1 year after harvest and no volunteers emerged at any site more than 2.5 years after harvest. However, OGTR monitoring data for three GM Indian mustard trial sites found that in two of the sites Indian mustard volunteers continued to emerge for about 5 years after harvest. Although these post-harvest trial sites were maintained in conditions conducive to germination of volunteers, there were three periods of 9-14 months where no volunteers were detected prior to reappearance of Indian mustard volunteers. This data suggests that Indian mustard seeds have greater dormancy in the seedbank than canola seeds. Therefore, for any trial site where Indian mustard is grown, the licence requires a monitoring duration of at least 36 months and until the site is free of volunteer plants for at least 18 months. If only canola is grown on a trial site, the required monitoring duration is at least 24 months and until the site is free of volunteer canola plants for at least 12 months.

165. The applicant proposes at least two post-harvest tillages of the trial sites to encourage seed germination. Tillage depth would be no greater than 5 cm, to avoid deep burial of seed that could induce dormancy. The first tillage would occur within 60 days after harvest and the final tillage would occur during the volunteer-free period prior to sign-off. To ensure that the final tillage produces conditions that are conducive to germination of volunteers, the licence requires this tillage to be followed by specified levels of rainfall or irrigation that provide sufficient moisture to the seedbank.

166. During the post-harvest monitoring period for each trial site, the applicant proposes to only plant crops permitted on GM brassica trial sites by the Regulator's Policy on Post-Harvest Crops. This will help to maintain the area in a manner appropriate to allow identification of volunteers.

167. The combination of control measures described in this section would minimise the persistence of GM seeds leading to establishment of GM volunteer populations in the environment (Risk Scenario 2).

3.1.6 Summary of licence conditions to be implemented to limit and control the release

168. A number of licence conditions are imposed to limit and control the release, based on the above considerations. These include requirements to:

- limit the duration of the release to the period from November 2022 to January 2028
- limit the size of the release to a maximum of twenty sites per year, with ten trial sites of up to 10 ha and ten trial sites of up to 5 ha
- limit the location of the release to nominated local government areas in NSW, Victoria and Queensland
- not allow GM plant material to be used in human food or animal feed, except for specified animal feeding experiments or taste testing experiments
- control pollen flow from the trial sites using one of the following options:
  (a) surround the planting area with a monitoring zone of 50 m and an isolation zone of a further 950 m
  (b) surround the planting area with a pollen trap of 15 m, a monitoring zone of 35 m and an isolation zone of a further 350 m
  (c) cover the planting area with an insect proof tent, and surround the planting area with a monitoring zone of 10 m and an isolation zone of a further 390 m
- treat any non-GM canola or Indian mustard grown in planting areas or pollen traps like the GMOs
- harvest the GM canola and Indian mustard separately from other crops
- clean equipment used with the GMOs before use for any other purpose
- transport and store the GMOs in accordance with the Regulator's guidelines
- locate planting areas and pollen traps at least 50 m from any natural waterways
- destroy all GMOs not required for further evaluation or future trials
- conduct post-harvest monitoring of the planting area and other areas where GM seeds may have been dispersed and destroy any volunteers that emerge
- on sites where any Indian mustard has been grown, monitor at least once every 30 days for at least 36 months after harvest and until the site is free of volunteers for at least 18 consecutive months
- on sites where only canola has been grown, monitor at least once every 35 days for at least 24 months after harvest and until the site is free of volunteers for at least 12 consecutive months
- conduct post-harvest tillage and irrigation of trial sites to encourage seed germination.

3.2 Other risk management considerations

169. 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

170. 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.

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

172. 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

173. 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 and Indian mustard outside permitted areas.

174. Before planting the GMOs, Nuseed must provide the Regulator with a method to reliably and uniquely detect the GMOs or the presence of the genetic modifications in a recipient organism.

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

175. The persons covered by the licence would be 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

176. 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.

177. 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 dates of cleaning after harvest
- details of inspection activities.

3.2.5 Monitoring for compliance

178. 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.

179. 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.

180. 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

181. Additional information has been identified that may be required to assess an application for a commercial release of these GM canola and Indian mustard lines, or to justify a reduction in limits and controls. The identified information is additional biochemical characterisation of the GM seeds with respect to levels of potentially toxic ω-3 LC-PUFA oxidation products and how these levels change over time.

Section 5 Conclusions of the RARMP

182. The RARMP concludes that the proposed limited and controlled release of GM canola and Indian mustard 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.

183. Conditions are 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 (2021). Australian Crop Report, No. 200, December 2021. (Canberra: Australian Bureau of Agricultural and Resource Economics and Sciences).

Albert, B.B., Cameron-Smith, D., Hofman, P.L., and Cutfield, W.S. (2013). Oxidation of marine omega-3 supplements and human health. Biomed Research International *2013*, 464921.

Albert, B.B., Vickers, M.H., Gray, C., Reynolds, C.M., Segovia, S.A., Derraik, J.G., Lewandowski, P.A*., et al.* (2016). Oxidized fish oil in rat pregnancy causes high newborn mortality and increases maternal insulin resistance. Am J Physiol Regul Integr Comp Physiol *311*, R497-504.

AOF (2019). Canola volunteer control 2019. (New South Wales, Australia: Australian Oilseeds Federation).

Arab-Tehrany, E., Jacquot, M., Gaiani, C., Imran, M., Desobry, S., and Linder, M. (2012). Beneficial effects and oxidative stability of omega-3 long-chain polyunsaturated fatty acids. Trends in Food Science & Technology *25*, 24-33.

Baker, J., and Preston, C. (2008). Canola (*Brassica napus* L.) seedbank declines rapidly in farmer-managed fields in South Australia. Australian Journal of Agricultural Research *59*, 780-784.

Brown, S. (2021). 2022 Victorian crop sowing guide. (Grains Research & Development Corporation, Agriculture Victoria).

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.

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

Christ, B., Hochstrasser, R., Guyer, L., Francisco, R., Aubry, S., Hörtensteiner, S., and Weng, J.-K. (2017). Non-specific activities of the major herbicide-resistance gene *BAR*. Nature Plants *3*, 937-945.

CSGA (2022). Canadian Regulations and Procedures for Pedigreed Seed Crop Production. (Canadian Seed Growers' Association).

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).

Gan, Y., Malhi, S.S., Brandt, S., Katepa‐Mupondwa, F., and Kutcher, H.R. (2007). *Brassica juncea* Canola in the Northern Great Plains: Responses to Diverse Environments and Nitrogen Fertilization. Agronomy Journal *99*, 1208-1218.

GRDC (2011). Break crop benefits: fact sheet. (Canberra, Australia: Grains Research & Development Corporation).

GRDC (2015). GRDC GrowNotes Canola - Southern Region. (Canberra, Australia: Grains Research & Development Corporation).

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

Gunasekera, C.P., Martin, L.D., Siddique, K.M.H., and Walton, G.H. (2006). Genotype by environment interactions of Indian mustard (*Brassica juncea* L.) and canola (*B. napus* L.) in Mediterranean-type environments 1. Crop growth and seed yield. European Journal of Agronomy *25*, 1-12.

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*, 1-14.

Hunter, K., Archer, D., Gesch, R., Vigil, M., Hatfield, J., Allen, B., Jabro, D.*, et al.* (2017). Degree Days to 50% Flowering for 12 Cultivars of Spring Canola-Like Mustard. Journal of Agriculture and Ecology Research International *11*, 1-8.

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.

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.

Kulkarni, S.S., Dosdall, L.M., Spence, J.R., and Willenborg, C.J. (2017). Brassicaceous Weed Seed Predation by Ground Beetles (Coleoptera: Carabidae). Weed Science *64*, 294-302.

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.

MacIntosh, S.C., Shaw, M., Connelly, M., and Yao, Z.J. (2021). Food and Feed Safety of NS-B5OO27-4 Omega-3 Canola (*Brassica napus*): A New Source of Long-Chain Omega-3 Fatty Acids. Frontiers in Nutrition *8*, 716659.

Matthews, P., McCaffery, D., and Jenkins, L. (2021). Winter crop variety sowing guide 2021. (NSW Department of Primary Industries).

McCaffery, D., Bambach, R., and Haskins, B. (2009). *Brassica juncea* in north-western NSW. (NSW Department of Primary Industries).

Miyashita, K. (2019). Prevention of Fish Oil Oxidation. J Oleo Sci *68*, 1-11.

O'Connor, S.E. (2017). Raising the BAR of specificity. Nature Plants *3*, 924-925.

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, Australia: Office of the Gene Technology Regulator).

OGTR (2013b). Risk Assessment and Risk Management Plan for DIR 117: Limited and controlled release of wheat and barley genetically modified for altered grain composition or nutrient utilisation efficiency. (Canberra, Australia: Office of the Gene Technology Regulator).

OGTR (2017). The Biology of *Brassica napus* L. (canola) and *Brassica juncea* (L.) Czern. & Coss. (Indian mustard). (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 and Risk Management Plan for DIR 164: Limited and controlled release of canola genetically modified for herbicide tolerance. (Canberra, Australia: Office of the Gene Technology Regulator).

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. Marine Biotechnology *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*. Metabolic Engineering *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.

Petrie, J.R., Zhou, X.R., Leonforte, A., McAllister, J., Shrestha, P., Kennedy, Y., Belide, S*., et al.* (2020). Development of a *Brassica napus* (canola) crop containing fish oil-like levels of DHA in the seed oil. Front Plant Sci *11*, 727.

Randall, R.P. (2017). A global compendium of weeds, 3rd edn (Perth, Western Australia: R. P. Randall).

Riar, A.S. (2015). Overcoming yield limitation of canola by improving water use efficiency. PhD Thesis (The University of Adelaide).

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.

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. Appl Microbiol Biotechnol *99*, 143-154.

Saini, R.K., Prasad, P., Sreedhar, R.V., Akhilender Naidu, K., Shang, X., and Keum, Y.S. (2021). Omega-3 Polyunsaturated Fatty Acids (PUFAs): Emerging Plant and Microbial Sources, Oxidative Stability, Bioavailability, and Health Benefits-A Review. Antioxidants (Basel) *10*.

Salisbury, P.A. (2006). Biology of *Brassica juncea* and potential gene flow from *B. juncea* to Brassicaceae species in Australia. (University of Melbourne).

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.

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.

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.

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

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.

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.

Virtue, J.G. (2008). SA weed risk management guide. (Adelaide: Government of South Australia: Department of Water, Land and Biodiversity Conservation).

Walsh, A.E. (2021). Economic implications of the loss of glyphosate and paraquat on central wheatbelt broadacre farms in WA. MSc Thesis (University of Western Australia).

Warwick, S.I., Francis, A., and Gugel, R.K. (2009). Guide to wild germplasm: *Brassica* and allied crops (tribe Brassiceae, Brassicaceae) 3rd edition.

Warwick, S.I., and Martin, S. (2013). Gene flow from transgenic oilseed *Brassica juncea* (L.) Czern. into weedy *Sinapis arvensis* L. (wild mustard). Plant Breeding *132*, 688-693.

White, M., Cheal, D., Carr, G.W., Adair, R., Blood, K., and Meagher, D. (2018). Advisory list of environmental weeds in Victoria. (Heidelberg, Victoria: Department of Environment, Land, Water and Planning).

Woodgate, J.L., Steadman, K.J., and Buchanan, K.L. (2011). A study of seed viability following consumption by birds. (Unpublished final report submitted to the OGTR).

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

Zarate, R., El Jaber-Vazdekis, N., Tejera, N., Perez, J.A., and Rodriguez, C. (2017). Significance of long chain polyunsaturated fatty acids in human health. Clin Transl Med *6*, 25.

Zhang, H., Skelton, A., Gardner, R.C., and Goddard, M.R. (2010). *Saccharomyces paradoxus* and *Saccharomyces cerevisiae* reside on oak trees in New Zealand: evidence for migration from Europe and interspecies hybrids. FEMS Yeast Res *10*, 941-947.

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 *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.

Appendix A: Summary of submissions from prescribed experts, agencies and authorities on the consultation RARMP

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

Submission	Summary of issues raised	Comment
1	Noted the recent flooding events in Queensland and NSW and suggested that assessing the risk of dispersal could be assisted by analysing GM canola and Indian mustard seed viability following a 1 to 2 week mock flooding study.	Chapter 1, Section 5.1 and Risk Scenario 2 (Chapter 2) discuss *B. napus* and *B. juncea* seed viability loss after prolonged exposure to water. Risk Scenario 2 in Chapter 2 and Section 3 of Chapter 3 of the RARMP discuss the likelihood of dispersal of the seeds by flooding. The licence conditions specify that planting areas and pollen traps must not be located in flood prone areas, that any extreme weather events must be reported to the Regulator, and that Planting Areas and Pollen Traps must be located at least 50 m from waterways.
	Otherwise, no concerns with the overall conclusions of the RARMP.	Noted.
2	Accepts that, overall, the application has negligible risks to the health and safety of people and the environment. Satisfied that measures taken to manage the short- and long-term risks from the proposal are adequate.	Noted.
3	Will the GMO crops be mechanically harvested? If so, will these machinery(s) be used solely to handle GMOs for the duration of the trial?	Harvesting may occur by hand or with commercial equipment (see Chapter 1 Section 5 and Chapter 3 Section 3). Specific licence conditions are included to restrict seed dispersal during growing and harvesting of the GMOs, including cleaning of any equipment before use for any other purpose.
	What are the risk management plans in the event of natural disasters, such as bushfires and floods?	Licence conditions have been imposed to restrict the likelihood of seed and pollen dispersal (including not planting in a flood prone area); to require a contingency plan that must be enacted, if required; and to notify the Regulator of any extreme weather events.
	In the event of an emergency, will emergency services personnel be aware that there are GMOs onsite?	The licence permits only authorised and trained trial staff to deal with the GM plants and their seeds. In the event of emergency personnel being onsite, case-by-case assessment and management would be conducted by the OGTR in consultation with the licence holder as soon as practicable.

Submission	Summary of issues raised	Comment
4	Advises that there is not sufficient data provided in the RARMP to support conclusions of negligible risks and recommends the following assessments be included in the RARMP:	
	Exposure of terrestrial organisms other than animals. Expand the organisms considered from just 'desirable animals' to 'other (desirable) organisms in the environment' particularly insects. Consider exposure of primary consumers, secondary consumers, and tertiary consumers as fatty acids may bioaccumulate in the biomass of organisms of higher trophic levels.	In the RARMP, the term 'desirable animals' includes insects. For clarity, wording has been amended to 'other desirable organisms.' Chapter 2, Section 2.4.1 (Risk Scenario 1) has been expanded to include soil organisms and pollinators. The limited size, duration, and controls in the field trial would restrict exposure and potential for harm, if applicable.
	Potential adverse impacts on beneficial organisms (e.g. insects). Cites a reference to indicate that the fatty acids may have detrimental effects on insects and cause deformed wings in insects. It is possible similar impacts may be seen in related beneficial insects.	Toxicity due to the altered oil content is discussed in Chapter 1 Section 4.3 of the RARMP. The referred study in the submission has been added to Risk Scenario 1. The limited size, duration, and controls in this limited and controlled field trial would restrict the number of beneficial organisms exposed to the GMO seeds.
	Potential beneficial impacts on pest organisms (e.g. rodents, rabbits, birds). The RARMP should discuss the potential for increased pest populations due to increased fitness or reproduction due to the fatty acids.	Text has now been included in Chapter 2, Section 2.2 of the RARMP. The limited size, duration, and controls in this limited and controlled field trial would restrict the number of pest organisms exposed to the GM seeds.
	Further consideration of the risk of seed dispersal by wind or endozoochory in birds. It is not clear what data can give confidence that endozoochory in birds will be at 'very low levels.' There are no controls to minimise seed dispersal by birds or wind.	Risk Scenario 2 in Chapter 2 discusses the likelihood of seed dispersal by birds and wind and has been amended for clarity. Licence conditions to minimise the likelihood of seed dispersal by wind have been imposed, and the limited size and duration of the field trial limits the potential for harm.
	Discussion of the uncertainty regarding survival and competitiveness of GM plants (especially GM Indian mustard) and hybrids with weedy relatives. Risk scenario 2 should clearly note the uncertainty and discuss further the potential for GM seed to survive and compete if dispersed outside the trial site	Risk Scenarios 2 and 3 in Chapter 2 consider the effects on the competitiveness of the GMO or hybrids of the GMO. Clarification added to Risk Scenario 2 in Chapter 2.
	The RARMP should discuss seed dispersal as a potential causal pathway for gene flow in Risk Scenario 3.	Seed dispersal is considered in Risk Scenario 2 and is therefore not included in Risk Scenario 3. Licence conditions are included to limit the likelihood of seed dispersal.

Submission	Summary of issues raised	Comment
5	The Regulator should further consider the wording around human sensory trials	Wording around human sensory trials has been amended in the RARMP. Licence Condition 21 has been amended for clarity.
	The Regulator should further consider the potential for outcrossing to commercial canola crops	Outcrossing to commercial canola crops is discussed in Risk Scenario 3. The potential for outcrossing and the effect of the limits and controls for the trial have been clarified in the text for this risk scenario.

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	Strongly objects to any genetic modification of food stuffs.	Noted.