



Australian Government

Department of Health

Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan For

DIR 149

Limited and controlled release of Indian
mustard (*Juncea canola*) genetically modified
for altered oil content

Applicant: Nuseed Pty Ltd

February 2017

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Summary of the Risk Assessment and Risk Management Plan for Licence Application No. DIR 149

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application for the limited and controlled release (field trial) of a genetically modified organism (GMO) into the environment. A Risk Assessment and Risk Management Plan (RARMP) for this application was prepared by the Regulator in accordance with the requirements of the *Gene Technology Act 2000* (the Act) and corresponding state and territory legislation, and finalised following consultation with a wide range of experts, agencies and authorities, and the public. The RARMP concludes that the field trial poses negligible risks to human health and safety and the environment and that any risks posed by the dealings can be managed by imposing conditions on the release.

The application

Application number	DIR 149
Applicant	Nuseed Pty Ltd (Nuseed)
Project title	Limited and controlled release of Indian mustard (<i>Juncea canola</i>) genetically modified for altered oil content ¹
Parent organism	Indian mustard [<i>Brassica juncea</i> (L.) Czern. & Coss.]
Introduced genes and modified traits	<ul style="list-style-type: none"> Seven genes involved in the biosynthesis of omega-3 fatty acids (altered oil content for human nutrition, animal nutrition and food processing)² One gene from a bacterium (selectable marker)²
Proposed location	Sites are to be selected from 102 possible local government areas in New South Wales, Victoria and Queensland
Proposed release size	4 sites in 2017 (maximum 2 ha per site), 10 sites in 2018 (maximum 5 ha per site) and 15 sites in each subsequent year (maximum 10 ha per site)
Proposed release dates	April 2017 – May 2022
Primary purpose	To evaluate the agronomic performance and oil profile of the GM Indian mustard under field conditions

Nuseed proposes to conduct a field trial with up to 50 lines³ of Indian mustard genetically modified to produce a high proportion of long chain omega-3 fatty acids (oils) relative to other fatty acids in the seed.

Risk assessment

The risk assessment concludes that risks to the health and safety of people, or the environment, from the proposed release are negligible. 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

¹ The title of the project as supplied by the applicant is ‘Limited and controlled release of *Brassica juncea* genetically modified for oil content’.

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

³ The term ‘line’ is used to denote plants derived from a single plant containing a specific genetic modification resulting from a single transformation event.

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 animals to the GM plant material, dispersal of GM seed leading to spread and persistence of the GMOs, and transfer of the introduced genetic material to sexually compatible plants. 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 GM plant material will not be used for human food or animal feed, the proposed limits and controls effectively contain the GMOs and their genetic material and minimise exposure; and the GM Indian mustard has limited ability to establish populations outside cultivation or transfer the introduced genetic material to other plants.

Risk management plan

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

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the 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 human food or animal feed, to minimise dispersal of GM seed or GM pollen from trial sites, to transport GMOs in accordance with the Regulator's guidelines, to destroy GMOs not required for testing or further planting, and to conduct post-harvest monitoring at trial sites to ensure all GMOs are destroyed.

Table of Contents

SUMMARY OF THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN	I
DECISION	I
THE APPLICATION	I
RISK ASSESSMENT	I
RISK MANAGEMENT PLAN	II
TABLE OF CONTENTS	III
ABBREVIATIONS	IV
CHAPTER 1 RISK ASSESSMENT CONTEXT	1
SECTION 1 BACKGROUND	1
SECTION 2 REGULATORY FRAMEWORK	1
SECTION 3 THE PROPOSED DEALINGS	2
3.1 The proposed limits of the dealings (duration, size, location and people)	2
3.2 The proposed controls to restrict the spread and persistence of the GMOs in the environment	4
SECTION 4 THE PARENT ORGANISM	5
SECTION 5 THE GMOs, NATURE AND EFFECT OF THE GENETIC MODIFICATION	6
5.1 Introduction to the GMOs	6
5.2 Introduction to fatty acid biosynthesis in plants	6
5.3 The introduced genes, encoded proteins and their associated effects	7
5.4 Toxicity/allergenicity of the proteins associated with the introduced genes	7
5.5 Phenotypic characterisation of the GMOs	8
SECTION 6 THE RECEIVING ENVIRONMENT	8
6.1 Relevant abiotic factors	8
6.2 Relevant agricultural practices	9
6.3 Presence of related plants in the receiving environment	9
6.4 Presence of similar genes and encoded proteins in the environment	10
SECTION 7 RELEVANT AUSTRALIAN AND INTERNATIONAL APPROVALS	10
7.1 Australian approvals	10
7.2 International approvals	10
CHAPTER 2 RISK ASSESSMENT	11
SECTION 1 INTRODUCTION	11
SECTION 2 RISK IDENTIFICATION	12
2.1 Risk source	12
2.2 Causal pathway	13
2.3 Potential harm	13
2.4 Postulated risk scenarios	14
SECTION 3 UNCERTAINTY	20
SECTION 4 RISK EVALUATION	21
CHAPTER 3 RISK MANAGEMENT PLAN	22
SECTION 1 BACKGROUND	22
SECTION 2 RISK TREATMENT MEASURES FOR SUBSTANTIVE RISKS	22
SECTION 3 GENERAL RISK MANAGEMENT	22
3.1 Licence conditions to limit and control the release	22
3.2 Other risk management considerations	26
SECTION 4 ISSUES TO BE ADDRESSED FOR FUTURE RELEASES	27
SECTION 5 CONCLUSIONS OF THE RARMP	28
REFERENCES	29
APPENDIX A SUMMARY OF SUBMISSIONS FROM PRESCRIBED EXPERTS, AGENCIES AND AUTHORITIES	33

Abbreviations

ALA	α -linolenic acid
ARA	Arachidonic acid
APVMA	Australian Pesticides and Veterinary Medicines Authority
CCI	Confidential Commercial Information under section 185 of the <i>Gene Technology Act 2000</i>
cm	Centimetres
DGLA	Dihomo- γ -linolenic acid
DIR	Dealings involving Intentional Release
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DPA	Docosapentaenoic acid
DPA n -6	Omega-6 docosapentaenoic acid
EPA	Eicosapentaenoic acid
DTA	Docosatetraenoic acid
ETA	Eicosatetraenoic acid
FSANZ	Food Standards Australia New Zealand
GLA	γ -linolenic acid
GM	Genetically modified
GMO	Genetically modified organism
GRDC	Grains Research and Development Corporation
ha	Hectare
HGT	Horizontal gene transfer
km	Kilometre(s)
LA	Linoleic acid
LC-PUFA	Long chain polyunsaturated fatty acid
LGA	Local government area
m	Metre(s)
μ mole	Micromole
NHMRC	National Health and Medical Research Council
NSW	New South Wales
OA	Oleic acid
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
PC2	Physical containment level 2
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
SDA	Stearidonic acid
the Act	The <i>Gene Technology Act 2000</i>

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.
2. The Act in conjunction with the Gene Technology Regulations 2001 (the Regulations), an inter-governmental agreement and corresponding legislation in States and Territories, comprise Australia’s national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.
3. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

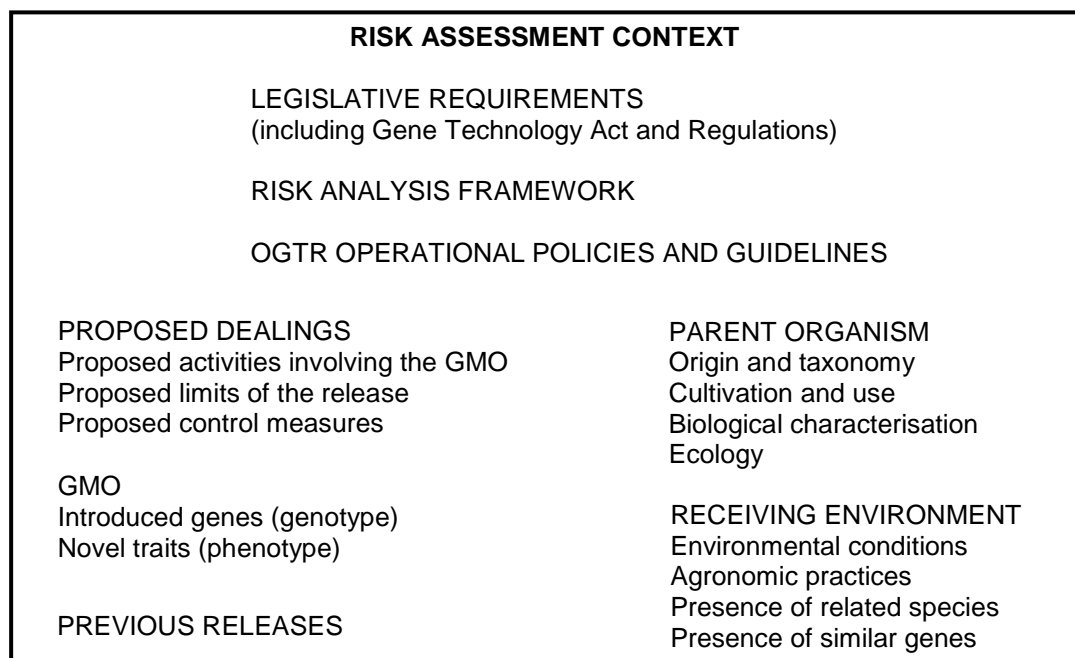


Figure 1. Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and who must be consulted, when preparing the Risk Assessment and Risk Management Plans (RARMPs) that inform the decisions on licence applications. In addition, the Regulations outline further matters the Regulator must consider when preparing a RARMP.
5. In accordance with section 50A of the Act, this application is considered to be a limited and controlled release application, as its principal purpose is to enable the applicant to conduct experiments and the applicant has proposed limits on the size, location and duration of the release, as well as controls to restrict the spread and persistence of the GMOs and their genetic material in the environment. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

6. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee, Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, relevant local council(s), and the public. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. No public submissions were received.

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

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

Section 3 The proposed dealings

9. Nuseed Pty Ltd (Nuseed) proposes to release up to 50 lines of Indian mustard (*Juncea canola*) genetically modified for altered oil content into the environment under limited and controlled conditions. The purpose of the release is to evaluate the agronomic performance and oil profile of the GM *Juncea canola* under Australian field conditions. Seed from the trial may be used for further seed increase or experimentation, including experimental animal feeding studies to assess nutritional qualities of the GM material.

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

- conducting experiments with the GMOs
- propagating the GMOs
- using the GMOs in the course of manufacture of a thing that is not a GMO
- growing the GMOs
- breeding the GMOs
- importing the GMOs
- transporting the GMOs
- disposing of the GMOs and
- possession, supply or use of the GMOs for any of the purposes above.

11. These dealings are detailed further below.

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

12. The release is proposed to take place over five years (from April 2017 to May 2022) at up to 4 sites for the first year, up to 10 sites in the second year and up to 15 sites in each subsequent year. Each site would be a maximum of 2 ha in the first year, up to 5 ha in the second year and up to 10 ha in each subsequent year.

13. The field trial sites may be located in 54 local government areas (LGAs) in New South Wales, 44 LGAs in Victoria and 4 LGAs in Queensland (Table 1). The exact site locations would be determined closer to planting, and their selection will depend on a number of factors including: the availability of water and land during a growing season; adequate site distribution across Australian Indian mustard growing areas; the ability to ensure isolation and containment; and the ability to

segregate from commercial Indian mustard/canola crops. Details of intended site locations would be provided to the Regulator prior to each planting season and, once notification of planting has been received, placed on the OGTR website.

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

New South Wales	Victoria	Queensland
Albury	Ararat	Lockyer Valley
Balranald	Ballarat	Southern Downs
Berrigan	Benalla	Toowoomba
Bland	Bendigo	Western Downs
Blayney	Buloke	
Boorowa	Campaspe	
Cabonne	Central Goldfields	
Conargo	Colac-Otway	
Coolamon	Corangamite	
Coonamble	Gannawarra	
Cootamundra	Geelong	
Corowa	Glenelg	
Cowra	Golden Plains	
Deniliquin	Hepburn	
Dubbo	Hindmarsh	
Forbes	Horsham	
Gilgandra	Indigo	
Griffith	Latrobe	
Gundagai	Loddon	
Gunnedah	Macedon Ranges	
Gwydir	Melton	
Harden	Mildura	
Hay	Mitchell	
Hume	Moira	
Jerilderie	Moorabool	
Junee	Mount Alexander	
Lachlan	Moyne	
Leeton	Murrindindi	
Liverpool Plains	Northern Grampians	
Lockhart	Pyrenees	
Mid Western	Shepparton	
Moree Plains	South Gippsland	
Murray	Southern Grampians	
Murrumbidgee	Strathbogie	
Muswellbrook	Surf Coast	
Narrabri	Swan Hill	
Narrandera	Towong	
Narromine	Wangaratta	
Orange	Warrnambool	
Parkes	Wellington	

New South Wales	Victoria	Queensland
Tamworth	West Wimmera	
Temora	Wodonga	
Tumbarumba	Wyndham	
Tumut	Yarriambiack	
Upper Hunter		
Urana		
Wagga Wagga		
Wakool		
Walgett		
Warren		
Warrumbungle		
Weddin		
Wellington		
Young		

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

15. Animal feeding experiments are proposed to be conducted with non-viable material from the GMOs, such as Juncea canola seed meal or oil. These might include studies involving rats, broiler chickens or fish. Approval from an animal ethics committee operating in accordance with the State and Territory legislation and National Health and Medical Research Council (NHMRC) codes of practice for animal experimentation would be obtained before conducting any animal experiments. Any such studies would be conducted by appropriately trained staff under controlled experimental conditions in research facilities. No animals from these studies would enter the human food supply or be released into the environment.

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

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

- locating trial sites at least 50 m away from natural waterways
- restricting access to trial sites to authorised staff
- restricting gene flow by controlling brassica weeds around the trial sites and adopting one of the following combinations of controls:
 - covering flowering GM Juncea canola with insect proof tents, surrounding each planting area with a 10 m monitoring zone and maintaining a 400 m isolation zone from other Indian mustard and canola crops
 - surrounding each planting area with a 15 m pollen trap of non-GM Juncea canola and a 50 m monitoring zone and maintaining a 400 m isolation zone from other Indian mustard and canola crops
 - surrounding each planting area with a 50 m monitoring zone and maintaining a 1 km isolation zone from other Indian mustard and canola crops
- ensuring that the 10 m or 50 m monitoring zone are kept free of related species
- harvesting the GM Juncea canola separately from any other crop
- cleaning trial sites and adjacent areas following harvest
- cleaning equipment before use for any other purpose
- destroying any plant material not required for further evaluation or seed increase

- post-harvest monitoring and destruction of any volunteer *Juncea* canola for 24 months and destroying any volunteer *Juncea* canola plants⁴
- transporting and storing GM material in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs (2011)*
- with the exception of feeding animals under approved experimental conditions, not allowing GM plant material or products to be used for human food or animal feed as part of this limited and controlled release.

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

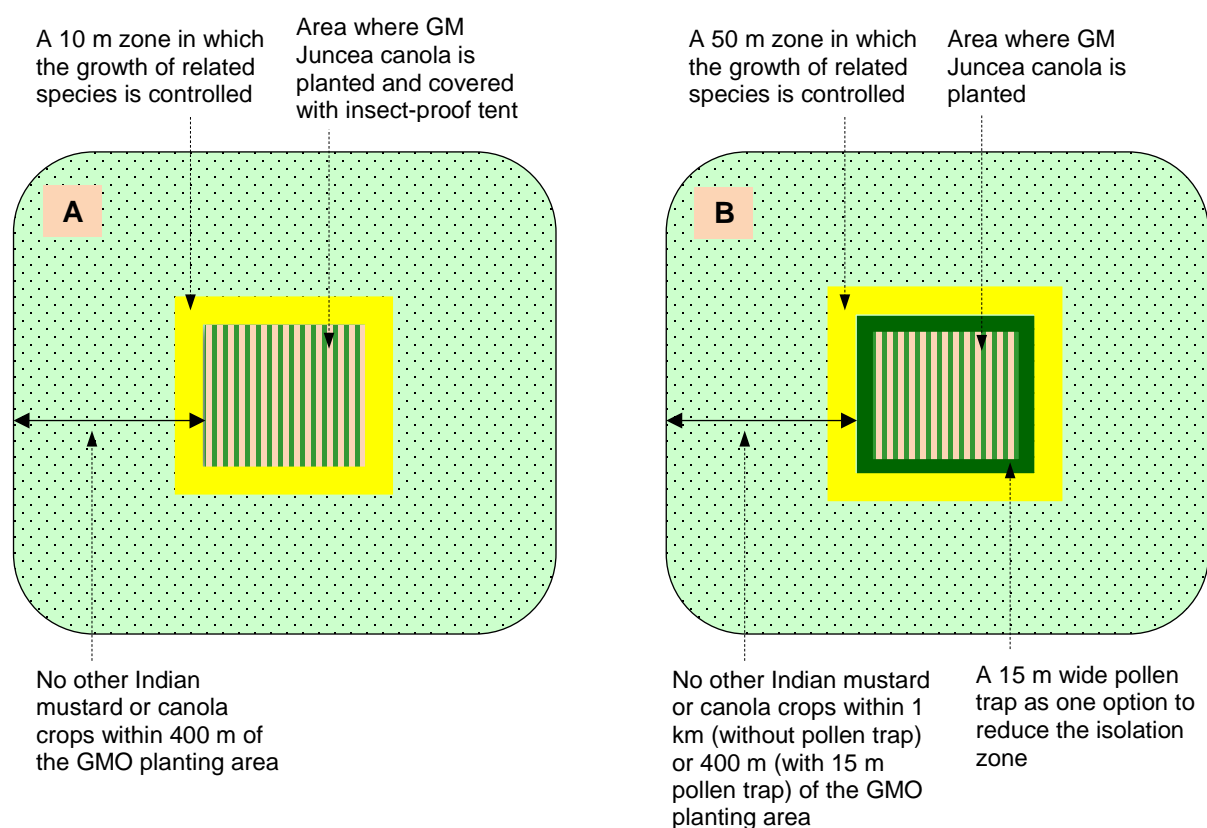


Figure 2. Proposed trial layout, including some of the controls (not drawn to scale).

A: controls when insect-proof tents are used; B: controls without insect-proof tents.

Section 4 The parent organism

18. The parent organism is *Brassica juncea* (L.) Czern. & Coss., which is commonly known as Indian mustard. It belongs to the Brassicaceae family and is exotic to Australia.

19. Indian mustard is cultivated worldwide as a condiment (mustard), an oilseed or a vegetable. Like rapeseed, Indian mustard naturally contains high concentrations of erucic acid and glucosinolates, the latter being responsible for the hot sensation of mustard.

20. Commercial Indian mustard production in Australia is on a small scale and is mainly in central New South Wales and western Victoria. In 2015, approximately 40,000 ha of Indian mustard were grown, mainly in NSW and representing less than 2% of the *Brassica* oilseed crop grown in Australia.

⁴ During the consultation of the RARMP, the applicant clarified their proposed post-harvest monitoring.

Indian mustard has greater tolerance to heat and water stress, greater resistance to blackleg disease, seeds with higher oil and protein content and is less prone to pod shatter than canola. Consequently, there is increasing interest in developing Indian mustard for Australian cropping environments, particularly as conventional breeding has led to the development of Indian mustard varieties that have low erucic acid and low glucosinolate content, enabling them to be considered “canola-quality” (Oram et al. 2005). This type of *B. juncea* is therefore referred to as *B. juncea* canola in the biology document referenced below or simply as Juncea canola by the industry. The GM lines included in this application are all developed from Juncea canola varieties and will be referred to as GM Juncea canola to distinguish it from *B. napus* canola, which is referred to simply as canola throughout this document.

21. Detailed information about the parent organism is contained in the reference document *The Biology of Brassica napus L. (canola) and Brassica juncea (L.) Czern. & Coss. (Indian mustard)* (OGTR 2016), which was produced to inform the risk assessment process for licence applications involving GM Indian mustard. Baseline information from this document will be used and referred to throughout the RARMP.

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

5.1 Introduction to the GMOs

22. The applicant proposes to release up to 50 lines of GM Juncea canola genetically modified for altered oil content.

23. The GM Juncea canola lines contain five to seven introduced genes involved in fatty acid biosynthesis pathways. These genes are expected to form a pathway to promote the accumulation of omega-3 fatty acids in the GM Juncea canola seed.

24. In addition to genes responsible for fatty acid biosynthesis, all GM Juncea canola lines also contain a selectable marker gene as well as short regulatory elements used to control gene expression. These sequences are derived from plants, soil bacteria and plant viruses.

25. Details of the introduced genes and regulatory elements have been declared CCI. The confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

26. The GM lines were produced using *Agrobacterium tumefaciens* mediated plant transformation. Information about this transformation method can be found in the document *Methods of plant genetic modification* available from the OGTR [Risk Assessment References page](#).

5.2 Introduction to fatty acid biosynthesis in plants

27. In commodity oil crops, oil is mainly synthesized during the maturation phase of the seed and primarily consists of triacylglycerols synthesized by esterifying glycerol-3-P with fatty acids (Tan et al. 2011). Indian mustard oil composition varies among different varieties but that of the Juncea canola is very similar to canola (*Brassica napus*), with the main components being oleic acid (OA, 57-63%), linoleic acid (LA, 18-25%) and α -linolenic acid (ALA, 8-13%) (Edwards & Hertel 2011).

28. In higher plants, long chain fatty acids are synthesized from shorter precursors derived from products of photosynthesis. Methods for introducing long chain polyunsaturated fatty acid (LC-PUFA, \geq C20) biosynthesis pathways into higher plants to produce long chain omega-3 fatty acids such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) have been extensively studied (Petrie & Singh 2011; Ruiz-Lopez et al. 2013). Biosynthesis of LC-PUFA occurs in nature by either aerobic or anaerobic pathways. Further discussion on this topic can be found in the RARMP for DIR 123 (OGTR 2013b).

29. Figure 3 shows a model of one aerobic pathway (the so called Δ 6-pathway) for biosynthesis of LC-PUFA in higher plants, which commences with the endogenous plant fatty acid oleic acid (OA). All the C18 fatty acids in this synthetic pathway up to γ -linolenic acid (GLA) in the omega-6 pathway and stearidonic acid (SDA) in the omega-3 pathway are commonly found in higher plants (Petrie & Singh

2011). The $\Delta 6$ -pathway has been shown to produce high levels of EPA and DHA in the oilseed crop *Camelina* (*Camelina sativa*) (Petrie et al. 2014; Ruiz-Lopez et al. 2014).

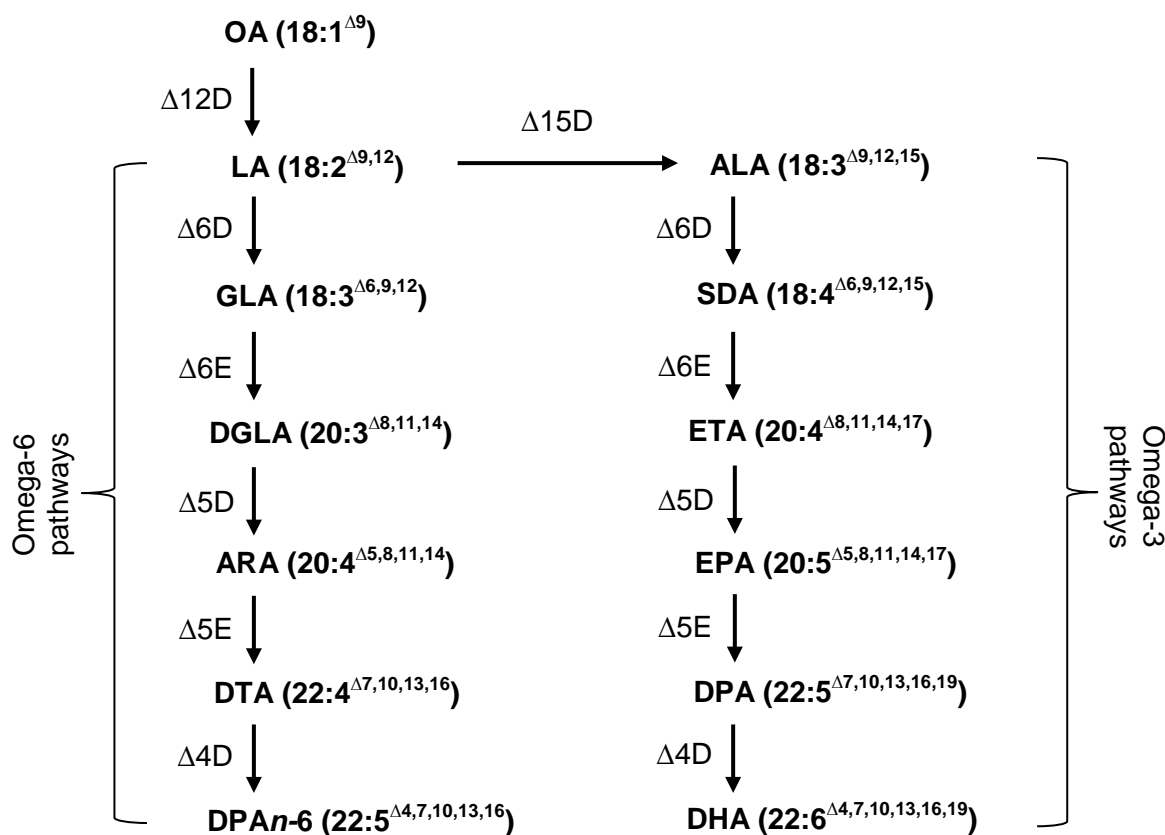


Figure 3. Long-chain polyunsaturated fatty acid biosynthesis pathways

Enzymes are referred to as either 'D' for desaturase (eg $\Delta 12D = \Delta 12$ -desaturase) or 'E' for elongase (eg $\Delta 6E = \Delta 6$ -elongase). The abbreviations for the fatty acids involved in the pathways are: ALA, α -linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; DGLA, dihomogamma-linolenic acid; DPA, docosapentaenoic acid; DPAn-6, omega-6 docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; ETA, eicosatetraenoic acid; GLA, γ -linolenic acid; LA, linoleic acid; OA, oleic acid; SDA, stearidonic acid.

5.3 The introduced genes, encoded proteins and their associated effects

30. The GM *Juncea canola* lines included in this application contain five to seven introduced genes involved in the biosynthesis of LC-PUFA depending on the transformation constructs used. All genes are codon optimised. Expression of these genes is expected to result in higher levels of omega-3 oils in the GM *Juncea canola* seed. All of the GM *Juncea canola* lines also contain a selectable marker gene from a bacterium. This marker gene was used in the early laboratory stages of development of the plants to enable selection of plant cells containing the desired genetic modification.

31. The identities of all of the introduced genes and molecular details of the constructs used for transformation have been declared CCI. The confidential information is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

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

32. The seven introduced genes involved in the novel LC-PUFA metabolic pathway were originally sourced from organisms not known to be toxic, allergenic or pathogenic to humans and other organisms. The source organisms of these genes have been declared CCI. The confidential information

is made available to the prescribed experts and agencies that are consulted on the RARMP for this application.

33. The LC-PUFA products of this biosynthesis pathway have been shown to promote human health and help prevent disease, and no toxicity or allergenicity has been associated with the consumption of these fatty acids (Wang et al. 2006; Whelan & Rust 2006).

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

35. Sequence similarity search of the introduced proteins against the Entrez Protein dataset from the National Center for Biotechnology Information using the BLASTP program (Altschul et al. 1997) revealed that none of the introduced proteins share sequence similarities with known protein toxins based on a cut-off E score of 1.0. The applicant also evaluated the degrees of similarity between each introduced protein product and allergenic proteins listed in the [AllergenOnline](#) database from the Food Allergy Research and Resource Program using the FASTA3 sequence alignment program (Pearson 2000). None of the introduced proteins had immunological relevant similarities with any of the known allergens in the database.

36. The selectable marker gene and its encoded protein have been extensively characterised and assessed as not posing a risk to human or animal health or to the environment by regulatory agencies in Australia and overseas.

37. No studies on the toxicity or allergenicity of the GM Juncea canola lines and their products have been undertaken to date as the proposed trial is at an early stage. Such studies may need to be conducted if approval was to be sought for any of the GM Juncea canola lines and their products to enter the human food supply chain.

5.5 Phenotypic characterisation of the GMOs

38. The GM Juncea canola lines are expected to express an enhanced omega-3 oil profile to accumulate specific LC-PUFA in the seed. None of the introduced genetic elements are known to affect other metabolic pathways within the *Brassica juncea* plant and the applicant states that preliminary observation of the GM Juncea canola plants grown in PC2 glasshouses did not indicate any unexpected phenotypic changes.

39. GM canola containing the same introduced genes for the LC-PUFA pathway has been field tested under DIR 123 (OGTR 2013b). The applicant reported that an enhanced omega-3 oil profile has been observed in the GM canola seed and there were no observable changes of agronomic performance of the GM canola plants grown under normal field conditions.

Section 6 The receiving environment

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

41. The proposed dealings involve planting of GM Juncea canola at up to 59 sites in both the winter and summer growing seasons between April 2017 and May 2022. These sites may be located in any of 102 LGAs in NSW, Victoria and Queensland (Table 1).

6.1 Relevant abiotic factors

42. The abiotic factors relevant to the growth and distribution of commercial Indian mustard in Australia are discussed in *The Biology of Brassica napus L. (canola) and Brassica juncea (L.) Czern. &*

Coss. (Indian mustard) (OGTR 2016). The proposed release will be carried out across a range of geographic and climatic conditions.

43. The applicant proposes to locate the GM *Juncea canola* trial sites at least 50 m away from natural waterways.

6.2 Relevant agricultural practices

44. The limits and controls of the proposed release are outlined in Sections 3.1 and 3.2. It is anticipated that the agronomic practices for the cultivation of the GM *Juncea canola* by the applicant will not differ significantly from industry best practices used in Australia, other than use of insect-proof tents. Plants at the release sites would therefore receive applications of water, fertilisers, pesticides and other agronomic management practices similar to commercially grown *Juncea canola* plants.

45. In Australia, Indian mustard is usually grown as a winter crop, with planting occurring in April or May and harvest in early summer. A summer crop can also be grown, with planting occurring in late spring/early summer and harvest in early autumn. Like canola, Indian mustard can be harvested either by direct heading or by windrowing (swathing). Windrowing involves cutting the crop and placing it in rows to dry. The windrow lies in horizontal bundles, supported by the cut stems 10 – 20 cm off the ground, and remains in the paddock for 8 to 19 days prior to harvest. When most of the seed has matured and the moisture content is 9% or less, the windrow is picked up by the harvester (GRDC 2010; Pritchard & Bluett 2008).

46. The GM *Juncea canola* would be grown from seed at field trial sites using conventional crop practices, and in some instances drip/pipe irrigation may be used to maintain the crop in dry weather where necessary. The applicant proposes to allow the GM *Juncea canola* to set seed and to harvest it separately from other Indian mustard or canola. Small areas/rows would be hand-planted or planted with a small plot seeder but larger areas would be planted with commercial equipment. Windrowing has been proposed and harvesting will occur either by hand threshing or with commercial equipment.

6.3 Presence of related plants in the receiving environment

47. The proposed trial sites are located in commercial Indian mustard and canola growing regions. As mentioned in Section 4, Indian mustard is currently only grown on a small scale in Australia, but with the release of non-GM Clearfield® *Juncea canola* varieties (DPI NSW 2013), the scale of planting may increase. Commercial canola varieties (both non-GM and GM) are grown on a large scale in New South Wales and Victoria. The GM canolas have been modified for herbicide tolerance. The applicant also requested to include non-GM canola (*Brassica napus*) varieties as comparators in the field trial⁵.

48. Indian mustard belongs to the Brassicaceae family, which consists of approximately 338 genera and over 3700 species worldwide (Warwick et al. 2006). Approximately 53 genera and 160 species are present in Australia, some of which are agriculturally important oilseed, vegetable or condiment crops and others are significant weeds (Jessop & Toelken 1986; Richardson et al. 2011).

49. As described in *The Biology of Brassica napus L. (canola) and Brassica juncea (L.) Czern. & Coss. (Indian mustard)* (OGTR 2016), *B. juncea* is self-compatible and mainly self-pollinating, but is capable of crossing with a limited number of other species. It can hybridise under natural conditions with *B. napus* (which includes canola), and gene flow to *B. napus* vegetables (swedes, rutabaga and kale) as well as forage rape is also possible. Outcrossing to *B. rapa* is also possible, but very much less likely than with *B. napus*; hybrids are characterised by a high level of male sterility and poor seed set (Salisbury 2006).

50. Significant pre- and post-fertilization barriers exist between Indian mustard and its weedy relatives in Australia. Gene movement between *B. juncea* and other wild relatives is rare, and in most cases probably never occurs (CFIA 2007). It is considered that, if such hybrids were to be produced

⁵ This request was made during the consultation period.

under natural conditions, their chance of survival would be extremely low (Salisbury, 2006). Naturally occurring field hybrids between *Juncea canola* and key Australian weeds *Raphanus raphanistrum* (wild radish), *Hirschfeldia incana* (Buchan weed) and *Sinapis arvensis* (charlock) have not been reported (Salisbury 2006). A study carried out in Canada also showed that the likelihood of introgression of traits from GM *B. juncea* to weedy *Sinapis arvensis* was low to negligible (Warwick & Martin 2013). More detailed discussion of *B. juncea* hybridisation can be found in the OGTR biology document (OGTR 2016).

6.4 Presence of similar genes and encoded proteins in the environment

51. The introduced genes and their encoded proteins are sourced from organisms that are widespread and prevalent in the environment. The selectable marker gene is from a bacterium also common in the environment.
52. Regulatory sequences are derived from plants, soil bacteria or plant viruses that are widespread in the environment. Although some of the regulatory sequences are derived from plant pathogens, they comprise only small parts of the total genomes and cannot of themselves cause disease.
53. The source organisms from which the introduced genes and regulatory sequences were derived have been declared CCI.

Section 7 Relevant Australian and international approvals

7.1 Australian approvals

7.1.1 Approvals by the Regulator

54. None of the GM *Juncea canola* lines included in this application have previously been approved by the Regulator for release in Australia. However, the Regulator has approved field trials of GM cotton (DIRs 039/2003 and 085/2008), GM safflower (DIRs 121 and 131) and GM canola (DIR 123) with modified oil content.
55. GM Indian mustard (*Juncea canola*) lines with herbicide tolerance and a hybrid breeding system have been approved for field trial in Australia under licences DIR 057/2004, DIR 069/2006 and DIR 104. There have been no reports of adverse effects on human health or the environment resulting from any of these releases.

7.1.2 Approvals by other government agencies

56. There are no approvals of these GM *Juncea canola* lines, including pending approvals, from other Australian authorities.

7.2 International approvals

57. None of the GM *Juncea canola* lines covered in this application have been approved for release in any other countries.

Chapter 2 Risk assessment

Section 1 Introduction

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

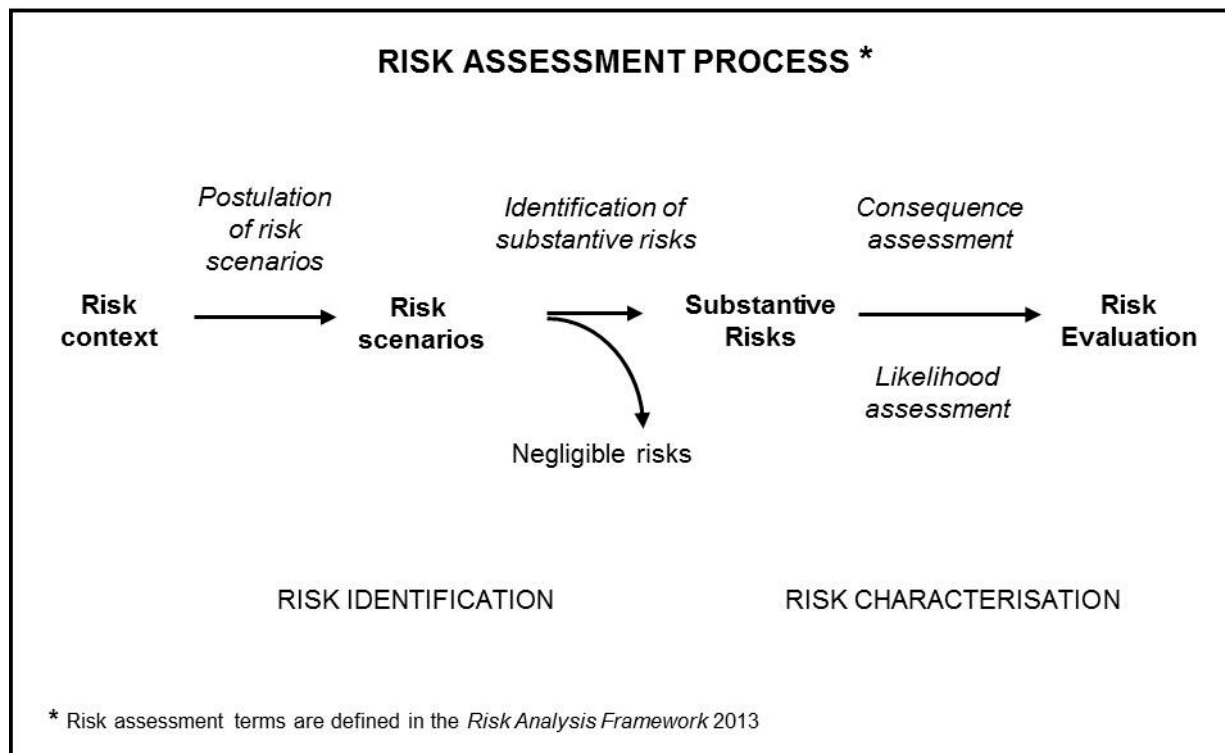


Figure 4. The risk assessment process

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

60. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, reported international experience and consultation (OGTR 2013a). A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants, 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. 2013). Risk scenarios postulated in previous RARMPs prepared for licence applications of the same or similar GMOs are also considered.

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

62. Substantive risks (ie those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood

assessment). 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

63. Postulated risk scenarios are comprised of three components:

- i. The source of potential harm (risk source).
- ii. A plausible causal linkage to potential harm (causal pathway).
- iii. Potential harm to an object of value, people or the environment.

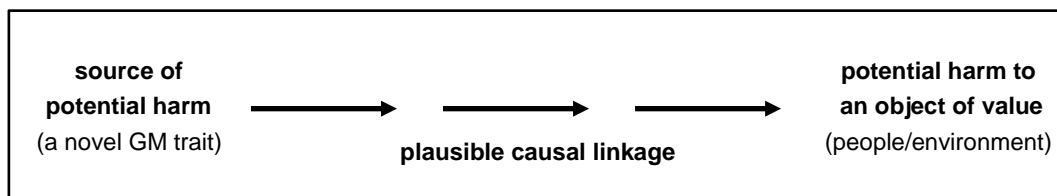


Figure 5. Risk scenario

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

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

2.1 Risk source

65. 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.

66. As discussed in Chapter 1, the GM Juncea canola lines have been modified by the introduction of up to seven genes for LC-PUFA production. These introduced genes are considered further as potential sources of risk.

Selectable marker genes

67. In addition, all of the GM Juncea canola lines contain a selectable marker gene. The identity of this gene has been declared CCI. However, this gene and its products have already been extensively characterised and assessed as not posing a risk to human or animal health or to the environment by regulatory agencies in Australia and overseas. Since the gene has not been found to pose risks to either people or the environment, its potential effects will not be further assessed for this application.

The regulatory sequences

68. The introduced genes are controlled by introduced regulatory sequences. The regulatory sequences are derived from plants, soil bacteria and plant viruses (see Chapter 1, Section 5.1). Regulatory sequences are naturally present in plants, and the introduced elements are expected to operate in similar ways to endogenous elements. The regulatory sequences are DNA that is not expressed as a protein, and dietary DNA has no toxicity (Society of Toxicology 2003). Hence, potential harms from the regulatory elements will not be considered further.

Unintended effects

69. The genetic modifications have the potential to cause unintended effects in several ways including altered expression of endogenous genes by random insertion of introduced DNA in the genome, increased metabolic burden due to expression of the introduced 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 safe use, and there are no documented cases where conventional breeding has resulted in the production of a novel toxin or allergen in a crop (Steiner et al. 2013). Therefore, the processes of genetic modification resulting in unintended effects will not be considered further.

2.2 Causal pathway

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

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

71. Although all of these factors are taken into account, some are not included in risk scenarios because they are regulated by other agencies or have been considered in previous RARMPs.

72. The potential for horizontal gene transfer (HGT) and any possible adverse outcomes has been reviewed in the literature (Keese 2008) and assessed in many previous RARMPs. HGT was most recently considered in the RARMP for [DIR 108](#). 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, HGT will not be assessed further.

73. Previous RARMPs have considered the potential for unauthorised activities to lead to an adverse outcome. The Act provides substantial penalties for unauthorised dealings with GMOs or non-compliance with licence conditions, and also requires the Regulator to have regard to the suitability of an applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, unauthorised activities will not be considered further.

2.3 Potential harm

74. Potential harms from GM plants include:

- harm to the health of people or desirable organisms, including toxicity/allergenicity
- reduced biodiversity through harm to other organisms or ecosystems

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

75. These harms are based on those used to assess risk from weeds (Keese et al. 2013; Standards Australia Ltd et al. 2006). Judgements of what is considered harm depend on the management objectives of the land where the GM plant may be present. A plant species may have different weed risk potential in different land uses such as dryland cropping or nature conservation.

2.4 Postulated risk scenarios

76. Three risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 2, and discussed individually below. Postulation of risk scenarios considers impacts of the GM *Juncea canola* or its products on people undertaking the dealings, as well as impacts on people and the environment if the GM plants or genetic material were to spread and/or persist.

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

Table 2. Summary of risk scenarios from the proposed dealings

Risk scenario	Risk source	Causal pathway	Potential harm/s	Substantive risk?	Reasons
1	GM <i>Juncea canola</i> expressing the introduced genes for LC-PUFAs	Cultivation of GMOs at trial sites ↓ Exposure of people who deal with the GMOs or of animals at the trial sites to encoded proteins and end products	Increased toxicity or allergenicity in people or increased toxicity to other desirable organisms	No	<ul style="list-style-type: none"> • The proteins encoded by the introduced genes occur naturally in the environment and are not known to be toxic or allergenic to people or toxic to other organisms. • The end products, omega-3 LC-PUFAs, are not toxic. • Plant material from the GMOs may be fed to animals in approved experiments but would not otherwise be used for human food or animal feed. • The limited scale, and other proposed limits and controls minimise exposure of people and other organisms to the GM plant material.

Risk scenario	Risk source	Causal pathway	Potential harm/s	Substantive risk?	Reasons
2	GM Juncea canola expressing the introduced genes for LC-PUFAs	Dispersal of GM seed outside trial limits ↓ GM seed germinates ↓ Establishment of populations of the GM plants	Increased toxicity or allergenicity in people or increased toxicity to other desirable organisms OR reduced establishment or yield of desirable plants	No	<ul style="list-style-type: none"> The genetic modifications of the GM Juncea canola lines are not expected to alter any characteristics associated with weediness. The limits and controls proposed for the release would minimise spread and persistence of the GM Juncea canola. The GM Juncea canola is susceptible to standard weed control measures. Risk scenario 1 did not identify toxicity, allergenicity or weediness of the GMOs as substantive risks
3	GM Juncea canola expressing the introduced genes for LC-PUFAs	Pollen from GM plants fertilises sexually compatible plants ↓ GM hybrid seed germinates ↓ GM hybrids spread and persist	Increased toxicity or allergenicity in people or increased toxicity to other desirable organisms OR reduced establishment or yield of desirable plants	No	<ul style="list-style-type: none"> The proposed limits and controls would minimise pollen flow to sexually compatible plants outside the trial sites. Risk scenarios 1 and 2 did not identify toxicity, allergenicity or weediness of the GMOs as substantive risks. Hybrids with sexually compatible plants are unlikely to differ.

Risk scenario 1

<i>Risk source</i>	GM Juncea canola expressing the introduced genes for LC-PUFAs
<i>Causal pathway</i>	<p style="text-align: center;">↓</p> <p style="text-align: center;">Cultivation of GMOs at the sites</p> <p style="text-align: center;">↓</p> <p style="text-align: center;">Exposure of people who deal with the GMOs or of animals at the trial sites to introduced proteins and end products</p> <p style="text-align: center;">↓</p>
<i>Potential harm</i>	<p style="text-align: center;">Increased toxicity or allergenicity in people or increased toxicity to other desirable organisms</p> <p style="text-align: center;">OR</p> <p style="text-align: center;">Reduced establishment or yield of desirable plants</p>

Risk source

78. The source of potential harm for this postulated risk scenario is GM Juncea canola expressing introduced genes for LC-PUFAs.

Causal pathway

79. Workers who cultivate, harvest, transport, experiment or conduct other dealings with the GM Juncea canola grown would be exposed to the GM plant material. As the applicant proposes that only authorised personnel can deal with the GM Juncea canola, other people are not expected to be exposed to the GM

plants or plant material. Potential pathways of exposure to the introduced proteins are ingestion, inhalation or dermal contact. There is little potential for exposure of the public to GM plant material as no GM plant material would be used for human food as part of this field trial.

80. Non-human organisms may be exposed directly to the introduced proteins through ingesting the GM plants, or exposed indirectly through the food chain, or exposed through contact with dead plant material (soil organisms). Other than a small number of animals that may be fed non-viable material from the GMOs under approved experimental conditions, livestock and other animals would not be expected to ingest the introduced proteins as the GM plant material is not to be used as animal feed and grazing of livestock around the trial sites is restricted by licence conditions.

81. As discussed in the RARMP for DIR 123, honeybees commonly use canola as a source of nectar and pollen. This is expected to be similar for Juncea canola. Therefore, nearby hives and the honey produced could contain GM Juncea canola pollen. However, the proposed isolation measures to limit gene flow through pollen movement will also minimise the likelihood of GM Juncea canola pollen occurring in honey. In addition, commercial procedures used for honey processing (eg sieving and filtering) will reduce the presence of GM Juncea canola pollen in honey. Canola pollen content in a range of canola honey samples from a diverse geographical areas in Australia ranged from 0.08% to 0.32% by dry weight (Hornitzky 2004).

82. At the end of the trial, the applicant proposes to destroy GM Juncea canola materials not required for further research purposes. In addition, the short duration and limited size of the proposed trial would limit the potential for exposure to the GM plant material.

Potential harm

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

84. Non-GM Juncea canola varieties in Australia contain very low levels of erucic acid (less than 1%) and glucosinolates (less than 30 μ moles/g) in the seed (OGTR 2016). These are similar to canola and are within the standard for canola oil (CODEX 2009; Oilseeds WA 2006). Occupational exposure to canola pollen, dust and flour have been implicated in allergic reactions in people and a number of putative allergens have been characterised, including seed storage proteins, but no allergic reaction to canola oil has been reported in people (OGTR 2016). No toxicity or allergenicity studies on the isolated proteins encoded by the introduced genes have been conducted by the applicant. However, as discussed in Chapter 1, Section 5.4 and Section 6.4, the introduced proteins are the same or similar to those present in organisms that are common and widespread in the environment and are not known to be toxic to humans or animals. Comparison of the encoded protein sequences to databases of known toxins and allergens did not indicate any significant similarity (Chapter 1, Section 5.4). Therefore, it is not expected that proteins encoded by the introduced genes will be toxic or allergenic, or lead to increased toxicity or allergenicity. In addition, GM canola containing the same introduced proteins has been field trialled under DIR 123 and no adverse effects have been reported.

85. The expected phenotypic difference between GM and non-GM Juncea canola would be the altered omega-3 oil composition of the seed. GM Juncea canola oil may contain various levels of the omega-3 LC-PUFAs, which are not naturally produced in non-GM Juncea canola (Chapter 1, Section 5.3). Omega-3 LC-PUFAs are part of the normal human diet, as they are common constituents of fish oils. A four-year clinical trial showed no identifiable risks associated with long-term consumption of DHA (Wheaton et al. 2003). Numerous studies have also revealed the benefits of DHA and other omega-3 LC-PUFAs to human health (Burdge & Calder 2005; James et al. 2003; Wang et al. 2006).

86. A recent study on American tree swallows showed that chicks fed omega-3 LC-PUFAs grew faster and had greater immunocompetence (Twining et al. 2016). If consumption of seed from the GM Juncea canola were to enhance the environmental fitness of pest animals such as rabbits, rats, mice or pest birds, this could lead to a greater impact of these animals on native or desirable vegetation. However, other food sources, such as aquatic insects (Twining et al. 2016), some seeds, leaves and nuts already contain omega 3

fatty acids and in agricultural cropping areas (such as those where the trial sites are located), food is not limiting for pest species. Thus the availability of additional omega 3 fatty acids through consumption of GM Juncea canola is unlikely to change the existing impact of known pest animals. In addition, the omega 3 fatty acids are only present in the GM Juncea canola seeds, so any effects will be limited to when seeds are present. The limits on the trial in both area and duration, and the controls to prevent dispersal also limit exposure and ensure that any effects will be similarly limited.

Conclusion

Risk scenario 1 is not identified as a substantive risk due to limited exposure and lack of toxicity or allergenicity of the introduced proteins or end products to humans, or toxicity to other desirable organisms or reduced establishment and yield of desirable plants. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Risk scenario 2

<i>Risk source</i>	GM Juncea canola expressing introduced genes for LC-PUFAs
<i>Causal pathway</i>	↓ Dispersal of GM seed outside trial limits ↓ GM seed germinates ↓ Establishment of populations of the GM plants ↓
<i>Potential harm</i>	Increased toxicity or allergenicity in people or increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable plants

Risk source

87. The source of potential harm for this postulated risk scenario is GM Juncea canola expressing introduced genes for LC-PUFAs.

Causal pathway

88. Potential dispersal of reproductive GM plant material outside the site boundaries would be limited to seed or pollen, as Juncea canola does not reproduce vegetatively under natural conditions. If GM Juncea canola seed was dispersed outside the trial sites or persisted at the sites after completion of the trial, the seed could germinate and give rise to plants expressing the introduced genes. These plants could spread and persist in the environment outside the trial limits and people and other organisms may be exposed to GM plant materials. Gene flow via pollen is discussed in Risk scenario 3.

89. Dispersal of GMOs outside the limits of the trial site could occur through the activity of people, including the use of agricultural equipment. Human activity is considered the most significant method of long-distance seed dispersal for canola as discussed in the RARMP for DIR 123 (OGTR 2013b). This would be the same for Juncea canola. The proposed trial sites would only be accessed by trained and authorised people. This will reduce inadvertent access by humans thus minimising dispersal of GM plant material. Dispersal of GM plant material by authorised people entering the proposed trial site would be minimised as the applicant proposes cleaning of all equipment used at the trial site, including clothing. All GM plant material would be transported in accordance with the Regulator’s transport guidelines, which will minimise the opportunity for its dispersal.

90. The activity of animals such as rodents, herbivores and birds could lead to dispersal of the GMOs outside the limits of the trial sites. Juncea canola seeds lack seed dispersal characteristics such as stickiness, burrs and hooks, which can contribute to seed dispersal via animal fur or feathers (Howe & Smallwood 1982). However, as discussed in the RARMP for DIR 123 (OGTR 2013b), very low numbers of viable canola seed may be transported by animals (such as birds, mice and sheep) through endozoochory (dispersal

through ingestion by animals). Since the Juncea canola seed is very similar to canola seed, it is expected that this route of dispersal would also be very similar.

91. Juncea canola seed lacks specialised structures that would assist their dispersal by wind. However, as discussed in Chapter 1, Section 6.2, Juncea canola may be windrowed prior to harvesting. Under very strong wind conditions, there is a possibility of dispersal of plant material from windrows to outside field boundaries. Post-harvest cleaning, as well as establishment of monitoring zones around trial sites, which are monitored during and after trials, will manage potential for dispersal of GM plant material.

92. It is also possible that flooding could transport GM plant material away from trial sites. The applicant has proposed to locate the trial sites at least 50 m from permanent natural waterways and man-made waterways that flow into natural waterways, and to choose sites not prone to flooding. This is considered sufficient to minimise the potential for seed dispersal during flooding.

93. Persistence of GMOs at the trial site could occur through dormancy of seeds in the seed bank. This could be managed through promoting germination of any residual GM Juncea seed by light post-harvest tillage and irrigation, and monitoring of the trial sites and destruction of Juncea canola volunteers for at least two years. These measures would minimise the likelihood of persistence of GMOs after completion of the trial.

Potential harm

94. The potential harms from this risk scenario are toxicity or allergenicity in people or toxicity to desirable organisms, or reduced establishment or yield of desirable plants.

95. As discussed in risk scenario 1, the introduced gene products and their end products are not expected to be toxic or allergenic to people or toxic to other organisms. Therefore, the GM Juncea canola is unlikely to have increased toxicity or allergenicity to people or increased toxicity to other organisms.

96. With respect to the potential for reduced establishment or yield of desirable plants, *B. juncea* is considered a weed of agricultural and disturbed habitats but is of minor significance to natural ecosystems in Australia (Groves et al. 2003). Although *B. juncea* is considered an invasive species in some countries such as China, Costa Rica and Mexico, it is not declared a noxious weed species in any country (Randall 2012). The GM Juncea canola could reduce the establishment or yield of desirable plants in agricultural settings if GM Juncea canola volunteers grew in other crops. If this happened, the GM Juncea canola volunteers could be controlled by standard measures such as application of herbicides or cultivation.

97. The GM Juncea canola could reduce the establishment of desirable plants in the natural environment if the GM Juncea canola spread and persisted as a weed in nature reserves, displacing native vegetation. However, the genotypes used for commercial Juncea canola cultivation are bred for maximum production in managed environments in which optimal water and nutrient availability is ensured. Juncea canola can tolerate drier and hotter conditions during flowering and pod fill compared to canola but its nutritional requirements for crop production are similar to canola (McCaffery et al. 2009). Therefore, in natural environments where water and nutrient availability are limited, Juncea canola is still a poor competitor compared with native species.

98. The only expected difference between GM Juncea canola and non-GM Juncea canola is altered fatty acid composition in the GM Juncea canola seed oil. It is possible that the altered seed oil composition may affect germination and survival of the GM Juncea canola seed/seedlings. The seed oils serve only as the primary energy source during germination and seedling growth, but seed oil content and the fatty acid composition of the seed oils play an important role in determining the fitness of the plants and may contribute towards plant adaptation (Sanyal & Decocq 2016). Therefore, changes of the fatty acid profile in the GM Juncea canola seeds to produce oil with LC-PUFAs have the potential to enhance their fitness and therefore make them more competitive. However, the fatty acids present in the GM Juncea canola seed are expected to have similar or less calorific values than those in non-GM Juncea canola due to the increased proportion of polyunsaturated fatty acids. It is therefore highly unlikely that the GM Juncea canola seeds will have any significant advantage over parental seeds in plant establishment. The applicant has indicated

that under glasshouse conditions, the GM Juncea canola proposed for release showed no phenotypic difference from non-GM parental Juncea canola.

Conclusion

99. Risk scenario 2 is not identified as a substantive risk due to the limited ability of Juncea canola to spread and persist outside cultivation, the proposed limits and controls designed to restrict dispersal of the GM Juncea canola, and the susceptibility of GM Juncea canola to standard weed control measures. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Risk scenario 3

<i>Risk source</i>	GM Juncea canola expressing the introduced genes for LC-PUFAs
<i>Causal pathway</i>	↓ Pollen from GM plants fertilises sexually compatible plants outside the trial sites ↓ GM hybrid seed germinates ↓ GM hybrids spread and persist ↓
<i>Potential harms</i>	Increased toxicity or allergenicity in people or increased toxicity to other desirable organisms OR Reduced establishment or yield of desirable plants

Risk source

100. The source of potential harm for this postulated risk scenario is the introduced genes for LC-PUFAs.

Causal Pathway

101. The first step in the causal pathway for this risk scenario is pollen from GM plants fertilising other sexually compatible plants. Juncea canola is predominantly self-pollinating, but up to 30% of *B. juncea* seeds can result from cross-pollination (OGTR 2016). Pollen can be transported by physical contact, wind or insect pollinators, chiefly honeybees, but gene flow studies have shown that outcrossing occurs at low levels and decreases rapidly with distance (OGTR 2016). It is not expected that the introduced genes for production of LC-PUFAs would alter the pollen dispersal characteristics of the GM Juncea canola.

102. As discussed in Chapter 1, Section 6.3, Juncea canola can hybridise under natural conditions with *B. napus* (which includes canola, *B. napus* vegetables and forage rape). However, Juncea canola is not sexually compatible with its common weedy relatives in Australia, which includes *H. incana*, *R. raphanistrum* and *S. arvensis*. Thus the most likely partners for hybridisation are commercial plantings of GM and non-GM canola, or non-GM Juncea canola.

103. The applicant has proposed a number of control measures (Chapter 1, Section 3.2) that would restrict the potential for pollen flow and gene transfer to sexually compatible plants. These include options of covering the flowering GM Juncea canola plants with insect-proof tents that prevent access by pollinators or planting a pollen trap of non-GM Juncea canola in combination with surrounding each trial site with a monitoring zones and/or isolation zones within which no Indian mustard and canola crops will be grown. These measures will reduce the likelihood of hybridisation occurring between the GM Juncea canola lines and compatible species.

104. The second step in the causal pathway for this risk scenario is exposure to, or dispersal of hybrid seeds containing the introduced proteins. The previous risk scenarios have discussed how the limits and controls proposed for the GMOs would minimise exposure (Risk Scenario 1), and minimise dispersal (Risk Scenario 2). The measures would also be effective for any hybrid seed.

Potential harms

105. In the unlikely event of gene transfer to a sexually compatible plant, it is possible that expression of the introduced genes could lead to toxicity or allergenicity in people or toxicity in desirable organisms, or reduced establishment or yield of desirable plants through increased spread and persistence of GM hybrids.

106. However, as discussed in Risk Scenario 1, the introduced gene products and their end products are not expected to be individually toxic or allergenic to people or toxic to other organisms and the GM Juncea canola itself is similarly unlikely to have increased toxicity or allergenicity to people or increased toxicity to other organisms. This is also expected to be the case if the introduced proteins are expressed in hybrids with non-GM Juncea canola or canola.

107. The potential for the GMOs to reduce establishment or yield of desirable plants was discussed in Risk Scenario 2. As noted there, although there is a potential for the GM Juncea canola to be more competitive due to the change in the seed oil content, it is highly unlikely that the altered seed oil composition will provide any significant advantage over parental seeds for germination and survival of the GM Juncea canola seeds. Similarly, the genetic modification is unlikely to provide a significant advantage to *B. napus*/Juncea canola hybrids. Therefore, GM hybrids expressing the introduced proteins are unlikely to show significantly greater spread and persistence in nature reserves or to survive standard weed management practices for Brassica volunteers in agricultural settings.

Conclusion

108. Risk scenario 3 is not identified as a substantive risk due to the limited ability of Juncea canola to outcross, and the proposed controls designed to restrict pollen flow from the GM Juncea. Further, risk scenarios 1 and 2 did not identify toxicity, allergenicity or weediness of the GMOs as substantive risks, nor are GM hybrids likely to differ in this regard. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

109. Uncertainty is an intrinsic property of risk analysis and is present in all aspects of risk analysis⁶.

110. There are several types of uncertainty in risk analysis (Bammer & Smithson 2008; Clark & Brinkley 2001; Hayes 2004). These include:

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

111. 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.

112. 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.

⁶ A more detailed discussion of uncertainty is contained in the Regulator's *Risk Analysis Framework* available from the OGTR website or via Free call 1800 181 030.

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.

113. For DIR 149, uncertainty is noted particularly in relation to:

- Potential increases in toxicity or allergenicity as a result of the genetic modification and
- Potential for increased spread and persistence of the GMOs, including in land uses outside of agriculture.

114. 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.

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

Section 4 Risk Evaluation

116. 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.

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

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

118. Three risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the control measures 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:

- none of the GM plant material or products will enter human food or animal feed supply chains
- the proteins encoded by the introduced genes and the end products are unlikely to be toxic or allergenic
- limited ability of the GM *Juncea* canola plants to establish populations outside cultivation
- limited ability of the GM *Juncea* plants to transfer the introduced genetic material to other plants
- limits on the size, locations and duration of the release proposed by Nuseed
- suitability of controls proposed by Nuseed to restrict the spread and persistence of the GM *Juncea* canola plants and their genetic material.

119. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM *Juncea* canola plants into the environment are considered to be negligible. The *Risk Analysis Framework*, which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no additional controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.

Chapter 3 Risk management plan

Section 1 Background

120. 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.

121. 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.

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

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

Section 2 Risk treatment measures for substantive risks

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

Section 3 General risk management

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

3.1 Licence conditions to limit and control the release

3.1.1 Consideration of limits and controls proposed by Nuseed

126. Sections 3.1 and 3.2 of Chapter 1 provide details of the limits and controls proposed by Nuseed in their application. These are taken into account in the three risk scenarios postulated for the proposed release in Chapter 2. Many of the proposed control measures are considered standard for GM crop trials and have been imposed by the Regulator in previous DIR licences. The appropriateness of these controls is considered further below.

127. The applicant proposes that the duration of the field trial would be confined to five years, with up to 4 trial sites during the first year, up to 10 sites for the second year and up to 15 sites for each subsequent

year. Each site would be a maximum area of 2 ha in the first year, 5 ha in the second year and 10 ha in each subsequent year. Sites are to be selected from 102 possible LGAs in NSW, Victoria and Queensland. The limited size and duration of the trial would limit the potential exposure of humans and other organisms to the GMOs (Risk Scenario 1).

128. Only authorised personnel with appropriate training would be permitted to deal with the GMOs. This measure would limit the potential exposure of humans to the GMOs (Risk Scenario 1) and accidental dispersal of the GMOs (Risk Scenario 2).

129. The applicant proposes to grow both GM *Juncea canola*, non-GM *Juncea canola* and non-GM *Brassica napus* in the trial sites. As non-GM *Juncea canola* or *B. napus* may be mingled with or fertilised by GM *Juncea canola*, a standard licence condition requires that all plants grown in a trial site must be treated as if they are GMOs. This will reduce the likelihood of dispersal of GM material (Risk Scenarios 1 and 2).

130. The applicant has proposed to use the same measures as those used for GM canola under DIR 123 to control pollen-mediated gene flow for GM *Juncea canola*, including the use of insect-proof tents, monitoring zones, isolation zones, and pollen traps. While no information is available regarding distances for pollen movement of *B. juncea* under field conditions in Australia (OGTR 2016), given the similarity in physiology it is expected that the pattern of *B. juncea* pollen movement would be very similar to *B. napus* (Salisbury 2006). Singhal et al. (2005) showed that, in India, no wind pollination occurred over a 40 m distance for *B. juncea*.

131. The applicant has proposed that all trial sites would be surrounded by monitoring zones in which sexually compatible species would be removed prior to flowering. The monitoring zones would be either 10 m or 50 m wide depending on whether or not the GM *Juncea canola* is contained in an insect-proof tent while flowering. As experimental evidence suggests that the rate of out-crossing is greatly reduced beyond 50 m from the pollen source, and no *Brassicaceous* weeds have been reported to hybridise with *Juncea canola* under natural conditions (Chapter 1, Section 6.3), a 50 m wide monitoring zone would restrict pollen-mediated gene flow via insects to other *Brassicaceous* species (Risk Scenario 3). If insect-proof tents are used, they will be placed over the GM *Juncea canola* at least seven days prior to expected flowering (based on field inspections) and remain in place until completion of the flowering period. This will minimise insect-mediated pollen movement to plants outside the trial site, which is mainly mediated by honey bees with some contribution by hoverflies (Langridge & Goodman 1975). Nonetheless, as the material used to make the tents is not completely pollen-proof, a low level of wind-mediated pollen dispersal might still be possible. Therefore, a 10 m monitoring zone is considered appropriate in conjunction with insect-proof tents.

132. The applicant has proposed that, if used, the pollen trap will be 15 m wide and comprised of non-GM *Juncea canola*. Pollen traps are an effective means of reducing pollen-mediated gene flow (Staniland et al. 2000) and are more effective at reducing gene flow than leaving the area barren (Morris et al. 1994; Reboud 2003). Pollen traps function by absorbing the majority of pollen dispersed by the wind or insect vectors. In the case of pollinating insects, the presence of pollen trap plants flowering synchronously with the GM *Juncea canola* may provide sufficient forage for incoming pollinating insects, reducing the need to visit the GM plants within. Alternatively, pollen trap plants may absorb the pollen deposited by visiting insects as they exit the trial site (Williams 2001).

133. The applicant has also proposed to maintain an isolation zone between the GM *Juncea canola* plants and any other Indian mustard or canola crops. The isolation zone would be 400 m from the outer edge of the pollen trap, if used, or from the planting area if insect-proof tents are used. If no insect-proof tent or pollen trap is used, the isolation zone would be 1 km from the edge of the area planted to the GMO.

134. The isolation distances proposed exceed those mandated for trials of GM canola overseas, which generally require an isolation distance of 50-400 m (Salisbury 2002). Moreover, they exceed the isolation distances required in Australia for the production of non-GM certified canola seed. Production of basic canola seed requires an isolation distance of 100 m from the nearest *Brassica* crop and the seed must contain no more than 0.3 % off-types, whereas production of certified seed requires an isolation distance of 200 m and must contain no more than 0.1 % off-types (Australian Seeds Authority Ltd. 2006; OECD 2008). Therefore, the proposed isolation zones and pollen containment measures are considered an effective

means of restricting pollen-mediated gene flow to any other Indian mustard or canola crops being grown for breeding, commercial or research purposes (Risk Scenario 3). However, as *Juncea* canola is still sexually compatible with a limited number of other Brassica species (see Chapter 1, Section 6.3), the licence also requires the GM *Juncea* canola to be isolated from crops of other sexually compatible species.

135. As discussed in Risk Scenario 2, human activities play the greatest role in spread of *Juncea* canola seed. There is potential for dispersal of seed during sowing, harvesting and threshing (mechanical dispersal). Sowing and harvesting activities may lead to dispersal of seed into the area immediately around the trial, including the monitoring zone. To minimise such seed dispersal, the applicant proposes to clean equipment used with the GMOs before using for any other purposes and to transport and store any plant material taken off-site for experimental analysis according to the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* (<http://www.ogtr.gov.au/>). These are standard protocols for the handling of GMOs to minimise exposure of people and other organisms to the GMOs (Risk Scenario 1), dispersal into the environment (Risk Scenario 2), and gene transfer (Risk Scenario 3). These cleaning and transport measures are included as licence conditions.

136. There is also a possibility of seed dispersal via movement of plant material under strong winds. As discussed in Risk Scenario 2, there is potential for dispersal of material from windrows in an unusually strong wind event, or under flooding conditions. A licence condition requires the licence holder to notify the Regulator in writing of the intended method of harvest for each trial site (eg hand harvesting, direct heading or windrowing). In addition, another licence condition requires the applicant to use appropriate measures to minimise likelihood of dispersal of windrowed plant material by wind or water. Appropriate measures may include: high density planting and growth of the *Juncea* canola prior to windrowing, ensuring that windrows are thick and heavy so as to minimise the likelihood of their movement off-site; cutting/windrowing to allow maximum stubble height, as longer stubble helps anchor the windrows; site selection to avoid flood or wind-prone areas; and/or use of a windrow roller, which has proven effective in forming tight, compact windrows that are resistant to wind. A further licence condition requires the applicant to provide details of the measures used to the Regulator.

137. The applicant proposes to clean the trial sites and adjacent areas after harvest by incorporating plant material into the soil for decomposition. During sowing and harvesting, plant material could be scattered into the area immediately surrounding the trial, so there is potential for residual seed to be present in both the trial site and the monitoring zone. In Risk Scenario 2 it was noted that during the period between harvest and cleaning, residual seed on the soil surface would be susceptible to dispersal by animal predation and water runoff after rainfall. Therefore, it is appropriate to require that cleaning occurs shortly after harvest. A licence condition requires that GMO planting areas, their associated monitoring zones and other areas where GM plant material may have dispersed must be cleaned within 14 days after harvest of the GMOs. The applicant has proposed burial of excess seed as one of the destruction methods. Deep burial of seed is considered an effective method of destruction, therefore conditions allowing deep burial, with requirements monitoring of burial sites, have been included in the licence.

138. The applicant proposes, in line with a standard DIR licence condition, that trial sites are located at least 50 m from natural waterways to minimise the chance of viable plant material being washed away from the sites. An additional licence condition has also been included requiring immediate notification of any extreme weather conditions such as strong winds or flooding, and of any movement of harvested plant material off the site. This would facilitate monitoring of the release by the Regulator and help to ensure that if any dispersal occurs it is appropriately managed.

139. The applicant proposes post-harvest monitoring of the trial site, pollen trap area and any areas used to clean equipment for 24 months, destroying any volunteer *Juncea* canola plants detected by hand or by herbicide application. Inspections are proposed on a monthly basis, conducted for the entire monitoring period. This is considered appropriate as germination patterns of volunteers can be variable (see discussion in the RARMP for DIR 114) and the period from germination to flowering can be less than 60 days. Therefore, the licence conditions require post-harvest monitoring at least once every 35 days for at least 24 months, and until no volunteers are observed in the most recent 12 month period. The 10 m or 50 m monitoring zone around the trial site would also be subject to this post-harvest monitoring. Records must be kept of monitoring activities and findings, including number and location of volunteers, which will allow

the Regulator to assess the ongoing suitability of these measures and provide additional information for future assessments.

140. The applicant also proposes measures to minimise the persistence of any GM *Juncea canola* plants and seeds in the seed bank at release sites after harvest of the proposed trial. These include measures to encourage germination such as tillage operations and watering if needed. As *Juncea canola* has very similar properties to canola in terms of seed germination and dormancy, these proposed measures are considered necessary for quickly reducing seed numbers in the seed bank. Therefore, licence conditions require two light tillage events for the planting area, pollen trap and at least 5 m beyond these areas, the first occurring no more than 60 days after harvest and another occurring in the 12 month volunteer-free period prior to cessation of monitoring for any site. The promotion of seed bank germination for GM canola was discussed in the RARMP for DIR 114. Tillage must not bury plant material to a depth of more than sowing and ideally would occur in conditions where germination of the GMO is reasonably likely to ensue (eg after irrigation or rainfall). These treatments would promote germination by ensuring any remaining seeds are placed at an appropriate depth in conditions that promote germination and will also encourage the microbial decomposition of any residual seed. The Regulator must also agree before monitoring may cease. These measures would minimise the persistence of the GMO in the environment (Risk Scenario 2).

141. In addition, a licence condition has been included to restrict grazing of livestock around trial sites. Grazing is only permitted in the monitoring zone after the planting area, pollen trap and monitoring zone have been cleaned, and only if the planting area, pollen trap and any other area within the monitoring zone where GMO may have been dispersed are surrounded by a fence at least 1 m high capable of excluding livestock.

142. The applicant does not propose that any of the GM plant material would enter the human food or animals feed supply, and the GM *Juncea canola* has not been assessed for food use by FSANZ. However, the applicant has indicated that they may feed some non-viable products (such as seed oil and meal) from GM *Juncea canola* generated from this trial to animals under experimental conditions. Approval from an Animal Ethics Committee operating under the NHMRC would be obtained before conducting any animal experiments. The Ethics Committees must also be provided with the final risk assessment and risk management plan prepared for application DIR 149 so that they are aware of the Regulator's assessment, including the risk context. Therefore, the licence includes a condition that prohibits the use of any material from the GMOs in human food or animal feed except as part of animal nutrition experiments. Additionally, animal experiments must have approval from an ethics committee. However, no licence conditions are imposed on the use of animals from the animal feeding studies as this will be for the Animal Ethics Committee to consider.

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

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

- limit the release to up to 4 locations in the first year, 10 locations in the second year and 15 locations in each subsequent year in nominated local government areas in New South Wales, Victoria and Queensland between April 2017 and May 2022
- limit each trial site to a maximum of 2 ha in the first year, 5 ha in the second year and 10 ha in each subsequent year
- locate the trial sites at least 50 m away from waterways
- restrict gene flow via pollen from field trial sites using one of the following measures:
 - cover flowering *Juncea canola* with insect-proof tents, surround each planting area with a 10 m monitoring zone and maintain a 400 m isolation distance between the planting area and other intentionally planted Indian mustard and canola crops
 - surround each planting area with a 15 m pollen trap of non-GM *Juncea canola* and a 50 m monitoring zone and maintain a 400 m isolation distance from other Indian mustard and canola crops

- surround each planting area with a 50 m monitoring zone and maintain a 1 km isolation distance from other Indian mustard and canola crops
- ensure that the 10 m or 50 m monitoring zone are kept free of related species
- harvest the GM *Juncea canola* plant material separately from other Indian mustard and canola crops
- clean all equipment used in connection with the GMOs as soon as practicable
- destroy all GM plant material remaining at trial sites after harvest
- apply measures to promote germination of any *Juncea canola* or canola seeds that may remain in the soil in and around the trial site, including at least two shallow tillage events
- monitor the sites for at least 24 months after harvest and until no volunteers are detected for a continuous 12 month period and destroy any *Juncea canola* or canola plants that may grow
- transport and store GM material in accordance with the Regulator’s guidelines
- with the exception of feeding animals under experimental conditions, not allow GM plant material or products to be used for human food or animal feed.

3.2 Other risk management considerations

144. 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 and
- access for the purpose of monitoring for compliance.

3.2.1 Applicant suitability

145. 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 and
- the capacity of the applicant to meet the conditions of the licence.

146. The licence includes a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

147. 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

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

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

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

150. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to growing the GMOs, Nuseed is also 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

151. 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 and
- any unintended effects of the trial.

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

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

3.2.5 Monitoring for compliance

153. 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 site.

154. 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.

155. 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

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

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

Section 5 Conclusions of the RARMP

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

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

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Appendix A Summary of submissions from prescribed experts, agencies and authorities⁷

Advice received by the Regulator from prescribed experts, agencies and authorities on the consultation RARMP is summarised below. 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.

Abbreviations:

Act: *the Gene Technology Act 2000*; **Ch:** Chapter; **DAFWA:** Department of Agriculture and Food, Western Australia; **FSANZ:** Food Standards Australia New Zealand; **GM:** genetically modified; **GTTAC:** Gene Technology Technical Advisory Committee; **LGA:** Local government area; **RARMP:** Risk Assessment and Risk Management Plan

Sub.No:	Summary of issues raised	Comment
1	<p>Council does not support the proposed field trial of GM Indian mustard in this LGA. Whilst the city does not produce crops of mustard, we are still of the view that there are risks of both an environmental and economic nature, that could damage various communities in Australia.</p> <p>Even with the control measures that restrict the spread and persistence of the GM plants and their introduced genetic material, there is still a chance of the spread of GM plants.</p> <p>Supports the view that Australia should be "green and pure" for the health and market advantages that can provide. Does not support the growing, storage and transport of GM crops within Australia that is in direct opposition to this marketing strategy. Not yet convinced that the release of GM products without significant direct benefits to public health should be permitted.</p>	<p>The Regulator has prepared a comprehensive RARMP, in accordance with the requirements of the Act, and includes a comprehensive and critical assessment of data supplied by the applicant, together with a thorough review of other relevant national and international scientific literature. The RARMP concluded that the controls are appropriate and that the trial poses negligible risk to the health and safety of people and the environment. Advice is also taken from experts, agencies and authorities prior to making the decision whether or not to issue a licence.</p> <p>Economic and marketing issues are outside the legislative responsibility of the Regulator, and are matters for the States and Territories.</p>
2	<p>Notes that a council resolution was passed in 2001 declaring the municipality to be a GM-free district.</p> <p>Council commitment to a clean and green image is demonstrated by the significant number of organic farmers in the region.</p> <p>Council cannot give any useful feedback on local issues without more details on the location of the release site, particularly in relation to areas of biodiversity or organic farms.</p>	<p>The RARMP concludes that risks to people and the environment from this field trial are negligible.</p> <p>Marketing issues, including declaring areas to be GM free for marketing purposes, are the responsibility of the states and Territories.</p> <p>The application is for a limited and controlled release and, apart from listing the LGAs, the exact site locations have not yet been decided. The risk assessment considers characteristics of the broad geographic regions where the release is proposed to occur. The draft licence requires the GPS coordinates of any sites planted to GMOs to</p>

⁷ Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment.

Sub.No:	Summary of issues raised	Comment
		be notified to the Regulator, which are then placed on the OGTR website.
3	Supports the application as the RARMP indicates that the proposed release poses negligible risks to people and the environment, and a range of licence conditions would ensure there is ongoing oversight of the release.	Noted.
4	Notes that the licence will prohibit the use of material from the trial for human or animal consumption.	Noted. The licence prohibits the use of material from the trial for commercial human food or animal feed. The applicant may conduct small scale animal feeding studies.
5	This is the first release of GM Juncea canola in Australia. Given the limits and control measures proposed, the risks to the environment are considered minimal.	GM Indian mustard (Juncea canola) with herbicide tolerance has been approved for field trial in Australia under licences DIR 057/2004, DIR 069/2006 and DIR 104.
	<p>Agrees that the introduced genes are unlikely to cause harm to other organisms from the limited and controlled release. However, agrees there is uncertainty regarding the toxicity of the plants as a result of the genetic modifications.</p> <p>The increased omega-3 fatty acids could also have indirect effects. For example, there may be beneficial effects in pest species (rabbits, rats, mice or pest bird) that may feed on GM Juncea canola seeds and result in improved growth and immunity. Such benefits in certain species may in turn lead to effects on other aspects of the ecosystem. This is an area of uncertainty which has not been discussed in the RARMP.</p>	<p>Risk scenario 1 discusses toxicity of the GM plants. The GM juncea canola will not enter the commercial food or feed supply and the introduced genes and proteins are not known to be toxic to humans or animals. The LC-PUFA products of this biosynthesis pathway have been shown to promote human health and help prevent disease, and no toxicity or allergenicity has been associated with the consumption of these fatty acids.</p> <p>Text has been added to Risk scenario 1 about the possible beneficial effects of the GM Juncea canola on pest species. The availability of additional omega 3 fatty acids through consumption of GM Juncea canola is unlikely to worsen the existing impact of known pest animals that are already well provisioned with oil seeds. In addition, the limits on the trial in both area and duration, and the controls to prevent dispersal also limit exposure of pest animals and ensure that any effects on ecosystems will be similarly limited.</p>
	<p>The RARMP may need to include further supporting information for assessing the weediness of GM Juncea canola.</p> <p>It appears that the Canadian experience of Juncea canola has been used in the RARMP to support the conclusion on the lack of competitiveness and weediness of the species. The information on weediness ranking of Juncea canola in Australia, used in the RARMP, is based on reports from over a decade ago.</p> <p>Juncea canola has blackleg resistance and higher resistance to drought and higher temperatures than canola and, therefore, under Australian conditions Juncea canola may be more competitive than canola and persist in natural ecosystems.</p>	<p>Discussion on the weediness of Juncea canola in the RARMP is mainly based on Australian experience (e.g. McCaffery et al. 2009) rather than Canadian experience. The weediness ranking of Juncea canola is based on the currently available information.</p> <p>The OGTR takes a comparative approach to risk assessment, using the parent organism as baseline. The current application is for a field trial of GM Juncea canola with altered oil content and the trait is unlikely to provide significant environmental advantage in comparison to the non-GM counterpart.</p>
	Agrees that the risk of gene flow to major Australian weeds is unlikely as there are strong	Noted. Chapter 1, Section 6.3, discusses the ability of

Sub.No:	Summary of issues raised	Comment
	<p>natural barriers to the integration of genes into weedy species. Gene transfer is more likely to occur with GM and non-GM canola and non-GM Juncea canola.</p> <p>Agrees that the trait of altered oil composition is unlikely to have any significant advantage to the recipient or hybrid plants, but notes some uncertainty about this conclusion (paragraph 97 of the RARMP).</p> <p>Recommends a more detailed discussion in the RARMP of the potential for this trait to provide a competitive advantage (i.e. increased plant fitness) and the risk of increased fitness in related species if gene transfer does occur.</p>	<p>Juncea canola to hybridise with other species and concludes that the most likely partners for hybridisation are commercial plantings of GM and non-GM canola, or non-GM Juncea canola.</p> <p>The applicant has proposed a number of control measures (Chapter 1, Section 3.2) that would restrict the potential for pollen flow and gene transfer to sexually compatible plants. These measures will reduce the likelihood of hybridisation occurring between the GM Juncea canola lines and compatible species.</p> <p>Some changes have been made in the RARMP in Risk Scenarios 2 and 3 in regards to the potential for increased plant fitness for GM Juncea canola.</p>
	<p>Agrees that many of the controls in place will effectively restrict dispersal such as monitoring zones, isolation zones, pollen traps, insect traps and destroying any seed.</p> <p>The RARMP (paragraph 134) indicates windrowing may be used in the proposed trial. However, the risk of dispersal of seed from windrows may warrant further assessment; recent research in Australia on GM canola demonstrates movement of plant material and dispersal by windstorm.</p> <p>The limits on the size (483 hectares), duration (5 years) and maximum number of sites (54) of the field trial may pose challenges for monitoring and compliance with licence conditions designed to restrict dispersal.</p>	<p>Noted.</p> <p>Windrowing is not usually used for harvesting Juncea canola seed in Australia, as Juncea canola is less prone to pod shatter. However, some farmers may still choose to use it. Therefore, the applicant wants to keep this option open for the licence in case windrow is used during the trial period. Nonetheless, the control measures mean it is unlikely that the GM Juncea canola will be dispersed into native bushland, even if windrowing is used. As discussed in Chapter 3, the licence requires that if windrowing is used the licence holder must minimise the likelihood of dispersal of the GMOs by wind or rain, and report these methods to the Regulator. Similar licence conditions were imposed for harvesting GM canola and Indian mustard by windrowing under licence DIR 104 and other canola licences, including DIR 123 and no issues have subsequently been reported to the Regulator. The Busi et al (2016) paper is concerned with material from commercial canola production which does not have these measures in place.</p> <p>This field trial is similar in size and duration to the previously approved field trial of GM canola under DIR 123. The Regulator has not observed or received reports of adverse effects from that trial, which commenced in 2013. The licence for DIR 149 includes a range of conditions relating to inspection and reporting obligations by the licence holder; the OGTR has monitoring and inspection program which effectively manages compliance with licence conditions.</p>
	<p>This is the first trial of GM Juncea canola in Australia and there has been no commercial release of GM Juncea canola anywhere in the world. With this background, and less</p>	<p>There have been a number of GM Juncea canola trials in Australia. Other GM Juncea canola (under the term of Indian mustard) lines with herbicide tolerance have been field</p>

Sub.No:	Summary of issues raised	Comment
	<p>information on this species than canola, there is a higher level of uncertainty with respect to risks from other GM plants that have been subject to multiple field trials.</p> <p>In particular, as noted in the RARMP, there is uncertainty in regard to the toxicity of the GM plant, and therefore appropriate data may be needed if a larger trial or commercial release is proposed.</p>	<p>trialled in Australia under licences DIR 057/2004, DIR 069/2006 and DIR 104. No adverse effects to human health or to the environment from the limited and controlled release of these GM <i>Juncea</i> canola lines have been reported.</p> <p>Noted. Ch 3 Sec 4 includes requirements for characterisation of the GM <i>Juncea</i> canola for any larger scale release.</p>
	<p>The potential beneficial effects to some pest species from the consumption of plant material containing omega-3 fatty acids is not likely to pose environmental risks with the proposed limited and controlled release. However, there is uncertainty in relation to environmental risks from such effects, if commercial release is proposed.</p>	<p>Noted.</p>
	<p>Further studies and data on risks due to persistence, competitiveness, gene transfer and weediness should be considered if a future commercial release is proposed. This would reduce uncertainty about the environmental risks and better inform both risk assessment and appropriate risk management measures.</p>	<p>Noted.</p>
6	<p>Agrees with the overall conclusions of the RARMP.</p>	<p>Noted.</p>
	<p>The Regulator should consider clarifying licence conditions that relate to use of non-viable plant material for animal studies.</p>	<p>The relevant licence conditions have been reassessed and text has been added to the RARMP for clarification.</p>
7	<p>Supports the conclusion that the proposed dealing poses negligible risk to human health and safety and the environment.</p>	<p>Noted.</p>
	<p>Concerns that the Biology Document and the RARMP omit a large volume of available information about Indian mustard's weediness in both Australia and overseas. Points out that the 2012 Global Compendium in the 2016 Biology Document lists over 100 references on <i>Brassica juncea</i>. Suggests that the Regulator consider updating the Biology Document and the RARMP to include a more balanced and rigorous statement of <i>B. Juncea's</i> potential as a weed and the risk it poses.</p>	<p>The RARMP has been amended to cite the 2012 Global Compendium of Weeds, and states from the available references that although <i>B. juncea</i> is considered an invasive species in some countries, it is not declared a noxious weed species in any country. This information has also been included in the Biology Document.</p>