Australian Government
Department of Health
Office of the Gene Technology Regulator

June 2022

Risk Assessment and Risk Management Plan (Consultation version) for

DIR 190

Commercial release of Indian mustard genetically modified for herbicide tolerance (RF3 juncea canola)

Applicant: BASF Australia Ltd

This RARMP is open for consultation until 3 August 2022.

Written comments on the risks to human health and safety and the environment posed by this proposed release are invited. You may make your submission

via mail to: The Office of the Gene Technology Regulator, MDP 54 GPO Box 9848, Canberra ACT 2601

or

via email to: ogtr@health.gov.au.

Please note that issues regarding food safety and labelling, the use of agricultural chemicals, and marketing and trade implications do **not** fall within the scope of these evaluations as they are the responsibilities of other agencies and authorities.

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Summary of the Risk Assessment and Risk Management Plan (Consultation Version)

for

Licence Application No. DIR 190

Introduction

The Gene Technology Regulator (the Regulator) has received a licence application for the intentional, commercial scale release of genetically modified (GM) Indian mustard in Australia. The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed release poses negligible risks to human health and safety and the environment. Licence conditions have been drafted for the proposed release. The Regulator invites submissions on the RARMP, including draft licence conditions, to inform the decision on whether or not to issue a licence.

The application

Application number	DIR 190
Applicant	BASF Australia Ltd (BASF)
Project title	Commercial release of Indian mustard genetically modified for herbicide tolerance (RF3 juncea canola)[1]
Parent organism	Indian mustard (*Brassica juncea* (L.) Czern. & Coss.)
Introduced genes and modified traits	• *bar* gene from *Streptomyces hygroscopicus* (for glufosinate tolerance) • *barstar* gene from *Bacillus amyloliquefaciens* (for restoration of male fertility)
Proposed locations	Australia-wide
Primary purpose	Commercial release for Indian mustard production

Risk assessment

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

The risk assessment process considers how the genetic modification and activities conducted with the GMO might lead to harm to people or the environment. Risks are characterised in relation to both the seriousness and likelihood of harm, taking into account information in the application, relevant previous approvals, current scientific knowledge and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP. Both the short and long term risks are considered.

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

[1] The title of the application submitted by BASF is "Commercial release of RF3 canola quality *B. juncea* in the Australia cropping system, genetically modified for herbicide tolerance".

The principal reasons for the conclusion of negligible risks are: the introduced proteins are not considered toxic or allergenic to people, or toxic to other desirable organisms; the parental GM canola line and other GM crops containing the introduced genes have a history of safe use in Australia and overseas; the introduced genes and proteins are widespread in the environment; the GM juncea canola and its progeny can be controlled using integrated weed management; the GM juncea canola is susceptible to the biotic or abiotic stresses that normally restrict the geographic range and persistence of juncea canola and the GM juncea canola has limited capacity to survive in natural habitats. In addition, food made from the GM juncea canola has been assessed and approved by Food Standards Australia New Zealand as safe for human consumption.

Risk management

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

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

As the level of risk is assessed as negligible, specific risk treatment is not required. However, the Regulator has drafted licence conditions regarding post-release review (PRR) to ensure that there is ongoing oversight of the release and to allow the collection of information to verify the findings of the RARMP. The draft licence, detailed in Chapter 4 of the consultation RARMP, also contains several general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements, which include an obligation to report any unintended effects.

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Abbreviations

ABARES	Australian Bureau of Agricultural and Resource Economics and Sciences
the Act	The *Gene Technology Act 2000*
ANZFA	Australia New Zealand Food Authority (now Food Standards Australia New Zealand)
APVMA	Australian Pesticides and Veterinary Medicines Authority
bar	Glufosinate tolerance gene from *Streptomyces hygroscopicus*
barnase	Male sterility gene from *Bacillus amyloliquefaciens*
barstar	Fertility restoration gene from *Bacillus amyloliquefaciens*
BBCH	Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie
CANBR	Centre for Australian National Biodiversity Research
CMP	Crop management plan
DAWE	Department of Agriculture, Water and the Environment
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic acid
DW	Dry weight
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
g	Gram(s)
GM	Genetically modified
GMO	Genetically modified organism
GRDC	Grains Research and Development Corporation
GT	Glyphosate tolerant
ha	Hectare
HBS	Hybrid breeding system
HGT	Horizontal gene transfer
IMI	Imidazolinone tolerant
ISAAA	International Service for the Acquisition of Agri-Biotech Applications
LOQ	Limit of quantification
LLOQ	Lower limit of quantification
µg	Microgram(s)
µmol	Micromole(s)
ND	Not determined
NSW	New South Wales
NZ	New Zealand
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
PAT	Phosphinothricin acetyltransferase

PCR	Polymerase chain reaction
PRR	Post release review
PubCRIS	Public Chemical Registration Information System Search (APVMA)
RAF	Risk Analysis Framework (2013)
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
RF	Fertility restoration
T-DNA	Transfer DNA
TT	Triazine tolerant
USA	United States of America
USDA-APHIS	United States Department of Agriculture - Animal and Plant Health Inspection Service

Chapter 1 Risk assessment context

Section 1 Background

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

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

3. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.

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

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

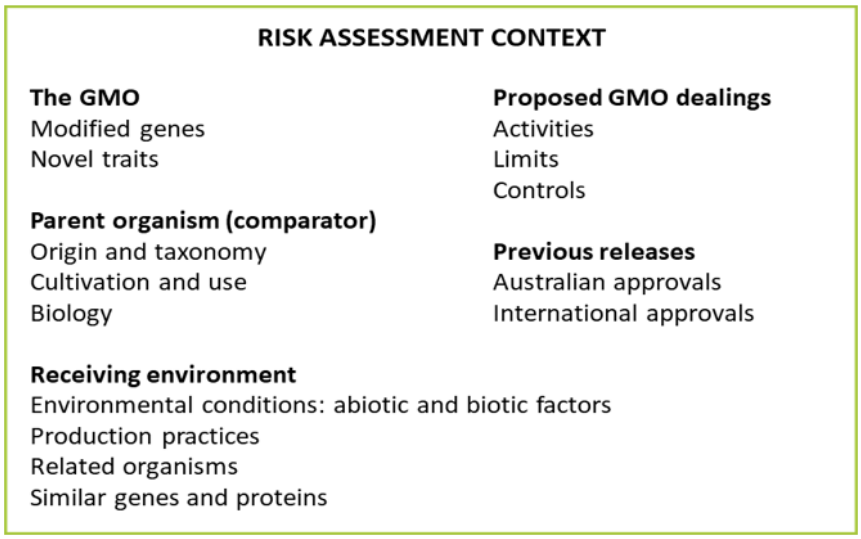

Figure 1 Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.

6. Since this application is for commercial purposes, it cannot be considered as a limited and controlled release application under section 50A of the Act. Therefore, under section 50(3) of the Act, the Regulator was required to seek advice from prescribed experts, agencies and authorities on matters relevant to the preparation of the RARMP. This first round of consultation included the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government

authorities or agencies prescribed in the Regulations, all Australian local councils[2] and the Minister for the Environment. A summary of issues contained in submissions received is provided in Appendix A.

7. Section 52 of the Act requires the Regulator, in a second round of consultation, to seek comment on the RARMP from the experts, agencies and authorities outlined above, as well as the public.

1.1 Interface with other regulatory schemes

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. The GMOs and any proposed dealings may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration, the Australian Industrial Chemicals Introduction Scheme and the Department of Agriculture, Water and the Environment (DAWE). These dealings may also be subject to the operation of State legislation recognising an area as designated for the purpose of preserving the identity of GM crops, non-GM crops, or both GM crops and non-GM crops, for marketing purposes.

9. To avoid duplication of regulatory oversight, risks that have been considered by other regulatory agencies would not be re-assessed by the Regulator.

10. FSANZ assesses the safety and nutrition of food produced using gene technology through administration of the *Australia New Zealand Food Standards Code.*

11. The DAWE regulates products imported into Australia to protect Australia from biosecurity risks. Under the *Biosecurity Act 2015*, the importation of biological material such as GM seeds requires a permit from DAWE.

12. Issues regarding herbicide use and resistance most appropriately fall under the *Agricultural and Veterinary Chemicals Code Act 1994*, and as such are the responsibility of the APVMA. The APVMA assesses all herbicides used in Australia and sets their conditions of use, including for resistance management.

Section 2 The proposed release

13. BASF Australia Ltd (BASF) proposes commercial cultivation of a genetically modified (GM) Indian mustard line, RF3 canola quality[3] *Brassic juncea* (RF3 juncea canola). RF3 juncea canola was developed by conventional breeding between the GM *Brassica napus* line RF3 (RF3 canola, also known by the OECD unique identifier ACS-BNØØ3-6) and a non-GM *B. juncea* line. It contains one introduced gene that confers tolerance to herbicides containing glufosinate and a gene for male fertility restoration, which is part of a hybrid breeding system (HBS). The applicant (BASF) states that although the introduced gene for male fertility restoration, part of the HBS, is present in RF3 juncea canola, it is their intention to use only the glufosinate tolerance trait for this commercial release.

14. The applicant is seeking approval for the release to occur Australia-wide, subject to any moratoria imposed by States and Territories for marketing purposes. RF3 juncea canola could be grown in all commercial canola growing areas or other areas suitable for juncea canola production, and products

[2] BASF is seeking approval for unrestricted commercial release of RF3 juncea canola in all canola growing areas, or other cropping areas suitable for juncea canola production in Australia and viable seed may be transported out of those areas. Therefore, the Regulator decided to consult with all local councils in Australia, except for those that have requested not to be consulted on such matters.

[3] Refer to Section 4 for the definition of 'canola quality'.

derived from the GM plants would enter general commerce, including use in human food and animal feed.

15. The dealings involved in the proposed intentional release are to:

(a) conduct experiments with the GMO

(b) breed the GMO

(c) propagate the GMO

(d) use the GMO in the course of manufacture of a thing that is not the GMO

(e) grow the GMO

(f) import the GMO

(g) transport the GMO

(h) dispose of the GMO

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

Section 3 Previous releases of the GM juncea canola proposed for release and other relevant GM canola

3.1 Australian approvals

3.1.1 GMO proposed for release

16. The RF3 juncea canola proposed for release has previously been approved for limited and controlled release into the environment (field trials) in Australia under the licences of DIR 057/2004, DIR 069/2006 and DIR 104.

3.1.2 Parental RF3 canola

17. The Regulator has previously authorised canola with the RF3 event for limited and controlled release under the licences of DIR 010/2001, DIR 069/2006 and DIR 104, as well as for commercial cultivation under the licence of DIR 021/2002[4].

3.1.3 Other relevant GM canola

18. A number of licences have been issued for commercial cultivation of GM canola with herbicide tolerance and HBS (Table 1). To date, the Regulator has not received any reports of adverse effects on human health, animal health or the environment caused by any releases of canola with the introduced herbicide tolerance and HBS traits.

Table 1 Previous approval of GM canola with introduced glufosinate tolerance and HBS for commercial release in Australia

DIR licence	Title	Included events	Modified traits
108	Commercial release of canola genetically modified for herbicide tolerance and a hybrid breeding system (InVigor® x Roundup Ready® canola)	MS8, RF3, GT73	Glufosinate and glyphosate tolerance, HBS

[4]The DIR 021/2002 licence authorises commercial release of GM canola lines containing the GM events including MS1, MS8, RF1, RF2, RF3, T45 and Topa19/2.

DIR licence	Title	Included events	Modified traits
138	Commercial release of canola genetically modified for dual herbicide tolerance and a hybrid breeding system (InVigor® x TruFlex™ Roundup Ready®)	MS8, RF3, MON 88302	Glufosinate and glyphosate tolerance, HBS
178	Commercial release of canola genetically modified for herbicide tolerance and a hybrid breeding system (MS11× RF3 and MS11 × RF3 × MON 88302)	MS 11, RF3, MON 88302	Glufosinate and glyphosate tolerance, HBS

3.2 Approvals by other Australian agencies

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

20. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has approved food derived from RF3 canola as safe for human consumption (ANZFA, 2001). FSANZ also approved RF3 juncea canola for food use through a notification of commercialisation in 2021, as RF3 juncea canola is the result of traditional breeding using a GM parent line for which food use has already been approved. As such it is not regulated as a new GMO (information provided by the applicant).

21. The APVMA has regulatory responsibility for agricultural chemicals, including herbicides, in Australia. The applicant holds a registration for the use of Liberty herbicide (glufosinate) for use on InVigor® hybrid varieties of canola (APVMA PubCRIS database, accessed April 2021). If RF3 juncea canola was approved for commercial cultivation in Australia, approval for the use of herbicides containing glufosinate in RF3 juncea canola crops would be needed.

3.3 International approvals

3.3.1 GMO proposed for release

22. BASF has obtained approval for food or feed use, or cultivation of RF3 juncea canola in Canada in 2020 and the USA in 2018 through notification[5] (information provided by the applicant).

3.3.2 Parental RF3 canola

23. A number of countries have approved the parental RF3 canola for commercial cultivation, as well as for food and feed use (Table 2).

Table 2 International approvals of RF3 canola

Country	Food	Feed	Cultivation
Canada	1997	1996	1996
China	2018	2018	
Columbia		2017	
EU	2013	2007	

[5] As the GM parent, RF3 canola, has been fully assessed and approved for food and feed use, and unconfined commercial cultivation in the USA and Canada, any progenies derived from it through conventional breeding, including RF3 juncea canola, are covered by these approvals and no further assessment is required.

Country	Food	Feed	Cultivation
Japan	2001	2003	2007
Mexico	2007		
New Zealand	2002		
Philippines	2018	2018	
South Korea	2013	2012	
Taiwan	2015		
USA	1998	1998	1999
Vietnam	2020	2020	

Source: ISAAA GM approval database; Biotrack Product Database; accessed February 2022

24. There have been no reports in the international literature of harm to human health and safety, or the environment, resulting from field trials or commercial cultivation of RF3 canola.

Section 4 The parent organism – non-GM *Brassica juncea*

25. As the GMO proposed for release is derived from conventional breeding between the GM *Brassica napus*, RF3 canola, and a non-GM *Brassica juncea* - juncea canola line 10CJ28-094 - two parent organisms are discussed in the following sections. This section provides information about the non-GM parent.

26. The non-GM 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. More detailed information regarding this parent organism can be found in the document *The Biology of* Brassica napus *L. (canola) and* Brassica juncea *(L.) Czern. & Coss. (Indian mustard)* (OGTR, 2017), which was produced to inform the risk analysis process for licence applications involving GM canola and juncea canola plants and is available from the Resources page on the OGTR website.

27. Indian mustard is cultivated worldwide as a condiment (mustard), an oilseed or a vegetable. Like rapeseed[6], Indian mustard seed naturally contains high concentrations of erucic acid and glucosinolates, the latter being responsible for the hot sensation of mustard.

28. Commercial production of Indian mustard in Australia is on a small scale, located mainly in central New South Wales and western Victoria. Indian mustard has greater tolerance to heat and water stress, greater resistance to blackleg disease and is less prone to pod shatter than canola. Consequently, there was interest in developing Indian mustard for Australian cropping environments, particularly as conventional breeding has led to the development of Indian mustard lines that have low erucic acid (<1% erucic acid in the seed oil) and low glucosinolate content (<30 µmol/g of glucosinolates in the seed meal), enabling them to be considered "canola quality" (Burton et al., 2003; Norton et al., 2009). 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 RF3 *B. juncea* included in this application is regarded canola quality by the applicant as it is developed by incorporating the RF3 trait from an RF3 *B. napus* line into an elite canola quality *B. juncea* line by conventional breeding 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.

[6] Rapeseed, also known as oilseed rape, refers to any *Brassica napus* crops that produce seed with a high content of erucic acid in the seed oil.

29. In establishing the risk context, details of the parent organism form part of the baseline for a comparative risk assessment (OGTR, 2013). Non-GM juncea canola is the standard baseline for biological comparison.

4.1 Indian mustard as a crop

30. Indian mustard is exotic to Australia and there are three types that Australian growers can grow: juncea canola, condiment mustard and industrial mustard (Norton et al., 2009). Juncea canola was only commercialised in 2007, but condiment mustard has been grown for a specific domestic niche market since the late 1980's (McCaffery et al., 2009b). Unlike canola, the scale of Indian mustard production in Australia has been very small and managed through a 'closed loop' marketing arrangement (Haskins et al., 2009). In 2015, Indian mustard cultivation reached approximately 40,000 ha, predominately in NSW (OGTR, 2017). However, the current planting areas have been estimated at less than 10,000 ha, due to the lack of new varieties with improved oil yield (McCaffery, 2022, personal communication).

31. Like canola, juncea canola seed is crushed to produce oil, which is used mainly as cooking oil or in food products. Juncea canola oil is also used in a range of industrial applications. The seed meal remaining after oil extraction is used as a high protein animal feed. Information on the use of the parent organism in agriculture is summarised in Section 7 (the receiving environment).

4.2 Weed risk potential for Indian mustard outside cultivation

32. *B. juncea* has been distributed worldwide as a crop, and has become naturalised in fields, wasteland and roadsides as a weed (Vélez, 2017). On a worldwide scale, information regarding the weediness of *B. juncea* is limited, indicating that it has not become a significant weed despite a long history of cultivation. In Canada, *B. juncea* is not considered a problem weed, which may be largely attributed to the small scale of cultivation (CFIA, 2012). In the USA, *B. juncea* is listed as a restricted noxious weed in Alaska and Michigan with allowable tolerances set for agricultural seed offered for sale and as an invasive weed in New Hampshire, but it is not present on the United States Federal Noxious Weeds list (Invasive.org, 2018).

33. *B. juncea* is naturalised in Australia. In areas where it is grown, it can be an agricultural weed in subsequent crops. There are isolated reports of *B. juncea* as an environmental weed in NSW and Victoria (Randall, 2017). However, the most recent Victorian state government environmental weed list gives *B. juncea* risk ranking score of zero, which means non-invasive and insignificant impact on natural systems (White et al., 2018). *B. juncea* is not recorded in the *Weeds of National Significance* list (Weeds Australia website, accessed February 2022), the *National Environmental Alert List* (Weeds Australia website, accessed February 2022), or the Noxious Weed List for Australian States and Territories (Invasive Plants and Animals Committee, 2015).

34. The weed risk of volunteer *B. juncea* has been assessed using methodology based on the *National Post-Border Weed Risk Management Protocol* (see Appendix 1, OGTR, 2017). This assessment protocol rates the weed risk of plants according to properties that correlate with weediness for each relevant land use (Standards Australia et al., 2006). These properties relate to the plants' potential to cause harm (impact), to its invasiveness (spread and persistence) and to its potential distribution (scale). For juncea canola, its actual, rather than potential, distribution is addressed. The relevant land uses considered were agricultural land use, intensive use areas such as roadsides, and nature conservation areas. A summary of the findings of the weed risk assessment are included in sections 4.2.1 to 4.2.3, below. For more detail, refer to Appendix 1 of *The Biology of* Brassica napus *L. (canola) and* Brassica juncea *(L.) Czern. & Coss. (Indian mustard)* (OGTR, 2017).

4.2.1 Potential to cause harm

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

- low potential to negatively affect the health of animals and/or people

- limited ability to reduce the establishment or yield of desired plants
- low ability to reduce the quality of products or services obtained from land uses
- moderate potential to act as a reservoir for pests or pathogens (OGTR, 2017).

36. *B. juncea* seeds contain two natural toxins: erucic acid and glucosinolates (OGTR, 2017). Erucic acid is found in the oil, and animal feeding studies have shown that traditional rapeseed oil with high levels of erucic acid can have detrimental health effects. Glucosinolates are found in the seed meal, which is used as livestock feed. The products of glucosinolate hydrolysis have negative effects on animal production (OECD, 2011).

37. The term *canola* refers to varieties of *B. napus*, *B. rapa* or *B. juncea* that contain less than 2% erucic acid in the oil and less than 30 µmol/g of glucosinolates in the seed meal, which are thus considered suitable for human and animal consumption (OECD, 2011).

4.2.2 Invasiveness

38. With regard to invasiveness, non-GM *B. juncea* has:

- the ability to reproduce by seed, but not by vegetative means
- short time to seeding
- high annual seed production in cropping areas
- low ability to establish amongst existing plants
- low tolerance to average weed management practices
- low ability to undergo long distance spread by natural means
- high potential for long distance spread by people and animals from cropping areas, and low potential for long distance spread by people and animals from intensive land uses such as roadsides (OGTR, 2017).

4.2.3 Actual distribution

39. In Australia, *B. juncea* is considered to be a weed primarily of agricultural or ruderal (disturbed) ecosystems, where it is considered to be a major problem warranting control (Groves et al., 2003).

40. Due to its primary colonising nature, *B. juncea* can take advantage of disturbed habitats such as roadside verges, field margins, wastelands and along railway lines. However, *B. juncea* is a poor competitor with weed species and do not establish well in unmanaged areas (Oram et al., 2005).

41. Feral *B. juncea* plants are often observed growing on roadsides or railway easements in Australia (Agrisearch, 2001). Like *B. napus*, roadside *B. juncea* populations are usually transient.

42. *B. juncea* is not considered a significant weed in natural undisturbed habitats in Australia, nor invasive of natural undisturbed habitats in Australia (Dignam, 2001; Groves et al., 2003). In the absence of disturbance, *B. juncea* is unable to compete with other plants and/or weeds and do not persist (OGTR, 2017).

4.2.4 Management of volunteer Indian mustard

43. Information regarding persistence of *B. juncea* volunteers is limited. However, fewer volunteers of Indian mustard than of canola have been observed in subsequent crops from field trials (Oram et al., 2005), possibly due to less pod shattering and seed loss during harvest of Indian mustard crop.

44. In Australia, the methods for control of canola volunteers also apply to Indian mustard (Australian Oilseeds Federation, 2019). This depends on the situation. When present in a fallow field, most control mechanisms are suitable, i.e. grazing, mowing, cultivation or herbicide application. When present in crops, control mechanisms are limited to herbicides and cultivation. Nine mode of action groups of registered herbicides (B, C, F, G, H, I, L, M and Q) are currently available to be used alone or in

combination for the control of Indian mustard volunteers. As for canola, volunteer Indian mustard is most easily controlled at the seedling stage.

Section 5 The GM parent – RF3 *Brassica napus*

45. The GM parental line for RF3 juncea canola is RF3 *Brassica napus* - canola containing the RF3 event (ACS-BNØØ3-6). RF3 canola has been extensively evaluated in previous RARMPs for both limited and controlled and commercial releases throughout Australia in the RARMP for DIR 021/2002, with reviews of this information in RARMPs for later commercial releases in which this event is also authorised (DIR 108, DIR 138 and DIR 178). As such, only a summary of the information about RF3 canola is provided here.

46. In establishing the risk context, details of the parent organisms form part of the baseline for a comparative risk assessment (OGTR, 2013). Non-GM juncea canola is the standard baseline for biological comparison, however, RF3 *B. napus* is also approved for commercial production and is the GM parent for the GMO proposed release, so information from RF3 *B. napus* is also relevant for purposes of comparative risk assessment.

5.1 The genetic modification of the parental RF3 canola

47. The introduced genetic material, source organisms and traits are summarised in Table 4.

Table 4 Introduced genetic elements

Gene (source)	Promoter (source)	Terminator (source)	Protein produced	Protein function
bar (*Streptomyces hygroscopicus*)	*PssuAt* (*Arabidopsis thaliana*)	*3' g7* (*Agrobacterium tumefaciens*)	PAT (phosphinothricin acetyl transferase)	Glufosinate tolerance
barstar (*Bacillus amyloliquefaciens*)	*PTa29* (*Nicotiana tabacum*)	*3'-nos* (*A. tumefaciens*)	Barstar (RNase inhibitor)	Restoration of male fertility

48. A detailed description of the genetic modification, including discussion of the genetic elements and the traits, is available in the RARMP for DIR 021/2002. This information was extensively reviewed in the RARMPs for DIR 138 and DIR 178. A summary of the information about this event will be presented here. RF3 canola was developed using *A. tumefaciens*-mediated transformation, a method used globally for introducing genes into plants.

49. In RF3 canola, a single insertion event occurred that resulted in the integration of two incomplete T-DNA copies arranged in an inverted repeat configuration. The exact location of the insert in RF3 canola is not known. More information is available in the RARMP for DIR 178.

50. In multiple field trials, breeding programs and seed production, there have been no reports of aberrant segregation or instability for RF3 canola.

5.1.1 The bar gene for glufosinate tolerance

51. RF3 canola contains the bialaphos resistance (*bar*) gene, isolated from *S. hygroscopicus* (Thompson et al., 1987), which was first assessed for commercial release in canola under DIR 021/2002 and has been reviewed in more recent RARMPs. The *bar* gene encodes a phosphinothricin acetyltransferase (PAT) protein that confers tolerance to glufosinate (Hérouet et al., 2005). PAT acetylates glufosinate, converting it to *N*-acetyl-L-glufosinate and rendering it inactive (OECD, 2002).

52. The *bar* gene introduced into RF3 canola was modified by a substitution of two N-terminal codons of the original bacterial gene (see RARMP for DIR 021/2002; Thompson et al., 1987; Rouan and De Both, 2018).

5.1.2 The barstar gene

53. RF3 canola also contains the *barstar* gene, derived from the common soil bacterium *Bacillus amyloliquefaciens*, and encodes an RNase inhibitor protein, Barstar. The *barstar* gene is controlled by the PTa29 promoter from tobacco (*Nicotiana tabacum*) that directs gene expression solely within the tapetal cell layer of the anthers. This gene has been used as a fertility restoration gene to work in conjunction with the RNase coding gene *barnase* (for male sterility) to form a HBS in GM canola (refer to the RARMP for DIR 178 for more detail). The *barstar* gene has no function without the complementary *barnase* gene. The current application does not include the *barnase* gene and as noted earlier there is no intent to use the HBS.

5.1.3 Toxicity/allergenicity of the proteins encoded by the introduced genes

54. RF3 canola has been approved for food and feed use as well as for environmental release in Australia and overseas with no credible reports of adverse effects (Section 3).

PAT protein

55. The *bar* gene and its encoded PAT protein have been extensively assessed in previous RARMPs for commercial release of GM crops including canola (DIR 021/2002, DIR 108, DIR 138, DIR 175 and DIR 178) and cotton (DIR 062/2005, DIR 143, DIR 145 and DIR 173). The PAT protein has been assessed to lack toxicity to humans or animals, or allergenicity in humans on the following basis:

- the *bar* gene was derived from the common, non-pathogenic soil bacterium *S. hygroscopicus*
- there is no sequence homology between PAT and known toxins or allergens
- the PAT protein has no characteristics associated with food allergens
- the PAT protein is inactivated by heat and by low pH
- high doses of PAT protein were not toxic to mice and rats in acute toxicity studies.

Barstar protein

56. Barstar is a ribonuclease inhibitor protein, which does not possess enzymatic activity. It instead exerts its action by binding to the Barnase enzyme to form an inactive complex when the *barnase-barstar* HBS is used. In the GMO proposed for release, the *barnase* gene is not present so the HBS is not active. The genes and proteins active in this system have been extensively assessed in previous RARMPs for commercial release of GM canola (DIR 021/2002, DIR 108, DIR 138, DIR 175 and DIR 178). The Barstar protein has been assessed to lack toxicity to humans or animals, or allergenicity in humans on the following basis:

- the *barstar* gene was obtained from the common, non-pathogenic soil bacterium *B. amyloliquefaciens*[7], which is used as a source of enzymes for food industries
- there is no sequence homology between Barstar and known toxins or allergens

[7] In 2018, *B. amyloliquefaciens* was added to the list of substances considered not to require control by scheduling in the Poisons Standard made under the *Therapeutic Goods Act 1989,* when used as a biofungicide. This is due to its low toxicity and ubiquitous presence in the environment.

- Barstar has no characteristics associated with protein allergens
- Barstar would not easily survive in the digestive tract
- feeding studies in animals have shown that canola lines containing Barstar (and Barnase) are nutritionally equivalent to non-GM canola.

57. FSANZ has approved food derived from RF3 canola expressing the Barstar protein as safe for human consumption (ANZFA, 2001).

58. FSANZ has approved food derived from a number of GM crops expressing the PAT protein as safe for human consumption. This includes GM canola (ANZFA, 2001; FSANZ, 2017), cotton (FSANZ, 2005a, 2010a, b, 2013), corn (FSANZ, 2005b) and rice (FSANZ, 2008).

5.1.4 Toxicity of glufosinate metabolites

59. The potential toxicity of herbicide metabolites is considered by the APVMA in its assessment of a new use pattern for particular herbicides, in this case glufosinate on RF3 juncea canola.

60. Herbicide metabolites produced in GM plants expressing PAT, following treatment with glufosinate, have been discussed in previous RARMPs for commercial release of GM crops including canola (DIR 021/2002, DIR 108, DIR 138, DIR 175 and DIR 178) and cotton (DIR 062/2005, DIR 143 and DIR 173). These RARMPs concluded that the main herbicide metabolites formed in GM plants following glufosinate treatment were less toxic than glufosinate and there is no suggestion that other metabolites produced by the activity of the PAT protein on endogenous plant amino acids (Christ et al., 2017) are toxic (O'Connor, 2017).

5.2 Toxicity/allergenicity of RF3 canola

61. The Regulator concluded in the RARMP for DIR 021/2002 that the InVigor® canola lines including RF3 canola are as safe as non-GM canola. This has been reviewed more recently in the RARMP for DIR 178. A summary of this information, including new or updated information since those RARMPs, is provided below.

62. Since the approval of RF3 canola, there have been no credible reports of adverse effects to humans, livestock or other organisms (Section 3).

5.2.1 Toxicity/allergenicity to humans

63. Canola oil is the only food product consumed by people, and oil from RF3 canola has been approved for human consumption in Australia (ANZFA, 2001) and other countries (Section 3).

5.2.2 Toxicity to animals including livestock

64. As discussed in previous RARMPs for canola (DIR 175 and DIR 178), canola can be used for feeding animals in the forms of unprocessed seed, seed meal and forage. Glucosinolates and erucic acid are naturally occurring toxicants in canola seed. Glucosinolates remain in the canola meal after oil extraction while erucic acid is removed with the oil fraction during processing of the seed. The levels of erucic acid and glucosinolates in the parental RF3 canola were below the limits specified in industry standards. RF3 canola is compositionally equivalent to non-GM canola varieties, with no significant differences other than the presence of the introduced proteins, and feeding studies on a range of animals demonstrate that there are no anti-nutritional effects of the genetic modification (ANZFA, 2001).

5.2.3 Toxicity to other organisms

65. A number of overseas regulatory agencies have assessed whether RF3 canola has any increased toxicity to non-target organisms as a result of the genetic modification. In its assessment of RF3 canola, the USDA-APHIS determined that it would not harm threatened or endangered species or other organisms, such as bees, that are beneficial to agriculture any more than conventional canola varieties

(USDA-APHIS, 1999). The Canadian Food Inspection Agency (CFIA) concluded that the unconfined release of RF3 canola would not result in altered impacts on non-target organisms, and that their potential impact on biodiversity is equivalent to that of currently commercialised canola varieties (CFIA, 1996).

66. The Barstar protein is only expressed in the tapetal cell layer during anther development when the gene is controlled by the tapetal cell specific promoter PTa29, and therefore, exposure is low.

5.3 Weediness of RF3 canola

67. The weediness of RF3 canola was assessed in the RARMP for DIR 021/2002 as posing negligible risk, and no credible reports of adverse outcomes as a result of the authorised release have been received (Section 3).

Section 6 The GMO proposed for release

6.1 Introduction to the GMO

68. As noted previously, the GMO proposed for release is RF3 juncea canola, derived from conventional breeding between the RF3 canola and a non-GM juncea canola line, 10CJ28-094.

The introduced genes and regulatory sequences for controlling the expression of the genes are shown in Table 4.

6.2 Characterisation of the GMO

6.2.1 Stability and molecular characterisation

69. Southern blot analysis was used to demonstrate the molecular equivalence of RF3 event in RF3 juncea canola to the same event in the RF3 canola using event-specific T-DNA probes (BASF, 2018b). Results from analysis of genomic DNA samples from five generations of RF3 juncea canola showed the insert organisation in RF3 juncea canola was identical to that of RF3 canola and stably inherited. Sequencing of the transgenic locus and its flanking sequences confirmed that no rearrangement occurred during conventional breeding (BASF, 2018a).

6.2.2 Levels of the introduced proteins in RF3 juncea canola

70. The applicant has supplied information on the expression levels of the PAT and Barstar proteins in whole plant, root, raceme and grain tissues collected from RF3 juncea canola plants across three generations, as determined by enzyme-linked immunosorbent assay (ELISA) (BASF, 2020a).

71. Plant tissue samples from RF3 juncea canola (both treated with glufosinate and untreated) were collected from field trials conducted at two sites in Canada and one site in the USA during the 2017 season. Levels of expressed proteins from the introduced genes were measured in plant tissues collected at growth stages of BBCH[8]14-16 (3-5 leaf), BBCH 30-39 (stem elongation), BBCH 57-65 (first flowering) and BBCH 87-99 (maturity), with five samples collected from single plants for each tissue type. Protein expression levels for tissues from glufosinate-treated plants are provided in Table 5. The data are shown as the arithmetic mean ± standard deviation (SD) and the range of values recorded as microgram (µg) of protein per gram (g) of tissue on a dry weight basis (dw). The means, SD, and ranges (minimum and maximum values) were calculated for each tissue type, with some sample values excluded from calculations when values are below the lower limit of quantification (LLOQ) or not available for analysis.

[8] BBCH growth stages, as described by Meier et al. (2009)

Table 5 Expression levels (µg/g dw) of introduced proteins in RF3 juncea canola

Tissue (Growth stage)	PAT Mean ± SD (range)	Barstar Mean ± SD (range)
Whole plant (BBCH 14-16)	117.06 ± 28.47 (71.34 – 167.49)	ND (<LLOQ – 0.08)
Whole plant (BBCH 30-39)	90.12 ± 35.00 (41.11 – 141.04)	ND (<LLOQ – 0.09)
Root (BBCH 30-39)	2.94 ± 1.87 (0.28 – 5.47)	ND (<LLOQ – 0.13)
Whole plant (BBCH 57-65)	58.89 ± 20.27 (28.56 – 84.15)	0.19 ± 0.05 (0.07 – 0.27)
Root (BBCH 57-65)	1.90 ± 1.03 (0.47 – 3.15)	ND (<LLOQ – 0.05)
Raceme (BBCH 57-65)	62.58 ± 12.01 (41.05 – 87.75)	0.66 ± 0.44 (0.04 – 1.32)
Grain (BBCH 87-99)	2.00 ± 1,38 (0.96 – 5.12)	ND (<LLOQ)

ND, not determined.

72. Expression of both PAT and Barstar proteins in RF3 Juncea canola showed very similar patterns to those of the parent line RF3 canola as shown in the RARMP for DIR 178, although concentrations of PAT were higher in RF3 juncea canola than those reported in RF3 canola in DIR 178.

73. Expression of PAT was measurable in all sampled plant tissues in RF3 Juncea canola. Generally, whole plant tissues (aboveground portion) from all stages showed high PAT expression with mean expression levels from 58.89 to 117.06 µg/g dw, while root tissues and grain samples had lower mean expression levels - at 2.94 and 2.00 µg/g dw, respectively. Floral tissues (raceme) also showed similar expression levels to the whole plant sample at the same stage.

74. Most RF3 juncea canola tissue samples had levels of Barstar expression below the LLOQ, with low levels only in the whole plant and raceme samples at flowering stage (BBCH 57-65) with mean values of 0.19 and 0.66 µg/g dw, respectively. This expression pattern is consistent with the fact that the *barstar* gene is controlled by the tapetum-specific promoter (Section 5.1.2).

6.2.3 Phenotypic characterisation and environmental interaction

75. Phenotypic characterisation (including agronomic characters) and environmental interaction data for RF3 juncea canola were collected from field trials conducted in canola growing regions in Canada and the USA during 2017 (BASF, 2020b). Twelve trial sites were selected that provided a range of environmental and agronomic conditions representative of the commercial canola production regions in Canada and the USA. These sites are within the agro-ecological zones (Fischer et al., 2021) that cover both rain-fed and irrigated cropping areas. Juncea canola can be grown in all canola growing areas in Australia that also include both rain-fed and irrigated land, and are located in all three Australian grains industry regions, comprising 13 agro-ecological zones. Some of these agro-ecological zones (e.g. temperate) are climatically similar to those of the selected trial sites in Canada and the USA. Also, like canola, juncea canola is a crop with a history of field trials, both in Australia and overseas, and the parameters for the agronomic and performance data used in the field trials in Canada and the USA were considered standard for data transportability (Garcia-Alonso et al., 2014). This study is therefore relevant to the Australian environment (Fischer et al., 2021). The trial sites provide comparisons of the GM juncea canola with its non-GM parental juncea canola and other conventional *B. juncea* varieties under climatic conditions similar to those experienced in Australia.

76. Both glufosinate treated and untreated RF3 juncea canola plants were included in the 2017 study. The parental juncea canola line, 10CJ28-094 (the control), was included as a non-GM control for all the phenotypic characterisation and environmental interaction studies, as well as the compositional analysis (Section 6.2.4), unless otherwise noted. In addition, seven commercial non-GM *B. juncea* varieties (four canola quality and three mustard quality) were also included as reference varieties to generate reference ranges for agronomic parameters for comparison. The reference range for each measured agronomic characteristic was determined from the minimum and maximum mean values from the seven reference *B. juncea* varieties planted among the sites. Comparison of RF3 juncea canola and the control was conducted within each site (individual site analysis) and in a combined-site analysis, in which the data were pooled across sites for agronomic characteristics. Data presented in Table 6 are from combined-site analysis and numbers represent sample means with SD. Statistical differences were identified at a 5% level of significance ($p<0.05$) for all data presented here.

Agronomic characterisation

77. Combined-site comparison of all agronomic parameters from glufosinate-treated RF3 juncea canola plants and the control is provided in Table 6. Statistically significant differences were detected for days to flowering, days to maturity and thousand seed weight. Similar results were obtained for RF3 juncea canola not treated with glufosinate, except that statistically significant differences detected only for days to flowering, days to maturity and plant height (data not shown). No statistical analysis was performed for plant lodging and pod shattering. All mean values for agronomic parameters for RF3 juncea canola were within the range of the reference varieties, indicating that RF3 juncea canola has no biologically relevant differences for the measured agronomic characteristics compared to conventional Indian mustard varieties.

Table 6 Combined-site analysis of agronomic parameters of RF3 juncea canola (glufosinate treated) and the control across all sites from the field trials in Canada and the USA during 2017

Parameter	RF3 Juncea canola Mean ± SD	Control Mean ± SD	Reference range[1]	p-value
Early stand count (plants/m^2)	139.15 ± 52.49	143.67 ± 40.07	54.55 – 345.83	0.613
Crop development (%)	78.1 ± 19.7	79.9 ± 19.2	10 – 100	0.335
Days to flowering	37 ± 3.9	36 ± 3.9	32 - 55	0.005
Flowering duration (days)	55 ± 11.3	55 ± 11.0	41 - 101	0.662
Final stand count (plants/m^2)	110.43 ± 44.35	120.19 ± 44.41	43.14 – 264.71	0.071
Plant height (cm)	118 ± 21.1	115 ± 19.0	82.2 – 193.6	0.088
Days to maturity	91 ± 9.5	89 ± 10.8	73 – 109	<0.001
Lodging (%)	6.7 ± 14.2	19.0 ± 23.3	0 – 90	NA[2]
Pod count	92 ± 53.0	89 ± 44.8	10.8 – 290.4	0.637
Pod shattering	5.18 ± 13.54	5.80 ± 17.86	0 – 131.4	NA[2]
Seed yield (T/ha)	2.50 ± 1.20	2.46 ± 1.05	0.007 – 4.576	0.822
Thousand seed weight (g)	3.22 ± 0.53	3.05 ± 0.42	2.07 – 4.12	0.048

[1] Range of results from seven reference varieties

[2] NA, not applicable (no statistical analysis performed due to limited variability of the data: lodging - 43.1% of the raw data based on individual plants had values of "0"; pot shattering - 55.1% of the raw data had values of "0")

78. The applicant also provided data from seed gemination and seed cold tolerance tests of RF3 juncea canola conducted in controlled plant growth chambers. In the seed germination tests, seeds were grown either at alternating temperatures of 20 ± 5°C for 16 hours and 30 ± 5°C for 8 hours per day for 6

consecutive days (warm germination test), or at 10 ± 5 °C for seven days and then incubated at alternating temperatures of 20 ± 5°C for 16 hours and 30 ± 5°C for 8 hours per day for 6 consecutive days (cold germination test) (Bayer, 2018b). In these warm and cold germination tests, both RF3 juncea canola and the non-GM control had germination rates of 99% and no statistically significant differences were detected. In the cold tolerance test, seeds were grown at -5 ± 5°C for 10 consecutive days followed by alternating temperatures of 20 ± 5°C for approximately 16 hours and 30 ± 5°C for approximately 8 hours per day for an additional seven days (Bayer, 2018a). Germination rates for both RF3 juncea canola and the control were approximately 25% and no statistically significant differences were detected. These results indicate that there is no significant difference in the germination potential of RF3 juncea canola compared to that of its non-GM parent.

Environmental interaction

79. Environmental interaction refers to the interaction between the crop plants and their receiving environment. The environmental interaction data collected in field trials in 2017 included plant response to abiotic stressors, disease and insect damage. At least three abiotic stressors, three diseases and three insect pests (arthropods) were evaluated four times during the growing season, at leaf development (BBCH 11-14), stem elongation (BBCH 31-39), flowering (BBCH 61-67) and pod development (BBCH 71-89). Comparisons between RF3 juncea canola and the non-GM control line, as well as three commercial non-GM *B. juncea* varieties (reference varieties), were carried out for each of the stressor categories across twelve field trial sites. The stressors selected varied from site to site as appropriate for individual site conditions, based on the environment of each site (BASF, 2020b).

80. Plant responses to abiotic stress, disease damage and arthropod damage was qualitatively assessed. The symptoms were assessed using a four-point qualitative rating scale: None (no damage), Slight (minor damage), Moderate (intermediate between Slight and Severe) and Severe (high damage). The abiotic stressors were selected from cold stress, drought, excess moisture, flood, hail injury, heat stress, nutrient deficiency, soil crusting and wind damage. Diseases were selected from Alternaria black spot, anthracnose, Aster yellows, black leg, bacterial leaf spot, clubroot, Downey mildew, Fusarium wilt, powdery mildew, Phytophthora root rot, Pythium, Rhizoctonia, Sclerotinia, seedling disease complex and wirestem. Arthropods included were alfalfa loopers, aphids, Bertha armyworms, cabbage worms, cabbage seedpod weevils, clover cut worms, diamond back moth, flea beetles, grasshoppers, Lygus bugs, red backed cutworms, red turnip beetles, root maggots and thrips.

81. A total of 425 comparisons between RF3 juncea canola and the control, or reference varieties, were carried out. No biologically relevant differences were observed between RF3 juncea canola and the control, or the reference varieties, for the selected stressors at any site. This indicates that the environmental responses of RF3 juncea canola were similar to those of its non-GM parent and other non-GM varieties.

6.2.4 Compositional analysis

82. The applicant provided data for compositional analysis of RF3 juncea canola seed harvested from eight field trial sites in canola growing regions in Canada and the USA during 2017 (BASF, 2020b), in comparison to the control and seven reference non-GM commercial *B. juncea* varieties. Compositional analyses include nutrients and anti-nutrients in grain (seed).

83. In this study, each entry (RF3 juncea canola, the control and reference varieties) was replicated four times in a randomised complete block design at each field trial. Compositional data from the reference varieties, was combined across all sites and used to calculate a 99% tolerance interval[9] for

[9] Tolerance interval: range of reference lines based on tolerance intervals specified to contain 99% of the population with 95% confidence.

each component to define the natural variability in commercial varieties. For any analytes where statistically significant differences ($p<0.05$) between RF3 juncea canola and the control were observed, mean values were compared to the tolerance interval, to assess whether the differences were likely to be biologically meaningful. Analytes with more than one third of sample values below the limit of quantification (LOQ) were excluded from statistical analysis. Only data for glufosinate-treated RF3 juncea canola are discussed here as this GM juncea canola is expected to be sprayed with glufosinate herbicides under the commercial production conditions.

84. Grain samples were analysed for analytes including proximates, fibre, amino acids, fatty acids, minerals, vitamins, and anti-nutrients. A total of 92 analytes were measured, but 25 of the analytes were not statistically analysed as more than one third of sample values for these analytes were below LOQ. These included 16 fatty acids, one type of vitamin E (β-tocopherol) and eight anti-nutrient glucosinolates. The remaining 67 analytes (54 nutrients and 13 anti-nutrients) were statistically assessed.

85. In the combined-site analysis, 22 of the 54 nutrient analytes showed no statistically significant difference between RF3 juncea canola and the control: three proximates, two amino acids, 11 fatty acids, four minerals, α-tocopherol and vitamin K1.

86. Statistically significant differences were identified in the other 32 nutrient analytes, with RF3 juncea canola having significantly higher levels in one proximate (crude protein), 16 amino acids (alanine, arginine, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, threonine, tyrosine and valine), four minerals (calcium, copper, magnesium and zinc) and three fatty acids (palmitic acid, heptadecenoic acid and linolenic acid), and significantly lower levels in one proximate (crude fat), two types of fibre (acid detergent fibre and neutral detergent fibre), one fatty acid (oleic acid), one mineral (potassium) and three types of vitamin E (γ-tocopherol, δ-tocopherol and total tocopherols). However, all these nutrient mean values were within the range of the reference varieties and tolerance intervals established by the reference varieties.

87. Among the anti-nutrients, no statistically significant differences between RF3 juncea canola and the control were identified in the combined-site analysis for 4-hydroxyglucobrassicin, gluconapin, neoglucobrassicin, phytic acid, sinapine, soluble tannins and total tannins. Statistically significant increases were identified in levels of glucobrassicin, gluconasturtiin, progoitrin, total glucosinolates and insoluble tannins. However, these anti-nutrient mean values were within the range of the reference varieties and the tolerance interval established by the reference varieties. In addition, no statistically significant differences between RF3 juncea canola and the control were identified in the combined-site analysis for erucic acid.

88. In summary, seed from RF3 juncea canola is compositionally comparable with seed from non-GM juncea canola varieties, and the observed differences in the seed component values between RF3 juncea canola and the control are not considered biologically meaningful from a food and feed perspective.

Section 7 The receiving environment

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

90. The applicant has proposed to release RF3 juncea canola in all agricultural cropping areas, including all canola growing areas, Australia-wide. Therefore, for this licence application, it is considered that the receiving environment is all of Australia, but in particular agricultural areas that are suitable to cultivate juncea canola. Juncea canola can be grown in most of the canola growing areas, with suitable production zones for Indian mustard located in New South Wales, Victoria, and South Australia (Norton

et al., 2009). The actual locations, number of sites and area of land used in the proposed release would depend on factors such as field conditions, grower demand and seed availability.

7.1 Relevant agronomic practices

91. In Australia, juncea canola is usually grown as a winter crop, with planting in April or May and harvest in early summer. A summer crop can also be grown, with planting in late spring/early summer and harvest in early autumn. Nutritional requirements for crop production are similar to canola (McCaffery et al., 2009a)

92. Unlike canola, juncea canola has natural pod shattering resistance which results in less seed loss at harvest and allows for direct combining (heading), eliminating the need for a swathing (windrowing) operation. However, some growers may still choose to windrow juncea canola crops for convenience (McCaffery et al., 2009a).

93. It is anticipated that agronomic practices for the cultivation of RF3 juncea canola proposed for release would not differ from standard industry practices. Glufosinate may be applied over the top of the GM juncea canola crop to control weeds, in the same manner that herbicides are applied over other herbicide tolerant canola varieties grown in Australia. Herbicides would be applied according to label directions approved by the APVMA. The APVMA assesses all herbicides used in Australia and sets their conditions of use. It should be noted that the Regulator will not consider issues relating to efficacy of the herbicide or resistance management as these issues most appropriately fall under the *Agricultural and Veterinary Chemicals Code Act 1994*, and as such are the responsibility of the APVMA.

94. The Crop Management Plan (CMP) for RF3 juncea canola, which farmers growing RF3 juncea canola would be required to follow, will be the same as that developed for RF3 canola. It will include guidelines for farmers on good hygiene to minimise the occurrence of off-types and volunteers during the production, handling, labelling, transport, and storage of GM and non-GM juncea canola, as well as on making appropriate herbicide choices for weed control.

7.2 Relevant abiotic factors

95. The geographical distribution of commercial juncea canola cultivation in Australia is limited by several abiotic factors. As juncea canola varieties generally have lower seed oil content than the best performing canola grown under the same conditions but are more drought and heat tolerant, they are more suitable to be grown in lower-rainfall zones when there is good subsoil moisture (Gunasekera et al., 2009; McCaffery et al., 2009a). However, moisture availability in soil is still one of the most important limiting factors for *B. juncea* crop yield, particularly during seed germination and seedling establishment (Mbatha and Modi, 2010). Germination of seed will only occur if there is sufficient soil moisture, and drought stress after anthesis can significantly reduce yield due to abortion of seed and reduced pod numbers (GRDC, 2009; OGTR, 2017). Like canola, juncea canola is sensitive to waterlogging (McCaffery et al., 2009a).

96. Frost can reduce juncea canola yields, particularly during early pod development and nutrient deficiency (OGTR, 2017). There is no information to suggest that *B. juncea* has different nutritional requirements to canola (McCaffery et al., 2009a). More detailed information regarding abiotic factors impacting the growth and distribution of *B. Juncea* in Australia is discussed in the reference document, *The Biology of* Brassica napus *L. (canola) and* Brassica juncea *(L.) Czern. & Coss. (Indian mustard)* (OGTR, 2017).

7.3 Relevant biotic factors

7.3.1 Presence of sexually compatible plants in the receiving environment

97. 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, while others are significant weeds (Jessop and Toelken, 1986; Richardson et al., 2011).

98. Gene transfer to sexually compatible plants in the receiving environment can occur via cross-pollination. *B. juncea* is self-compatible and mainly self-pollinating, but is capable of crossing with a limited number of other species (OGTR, 2017). 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* can occur, 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). Brassica vegetables are generally harvested prior to flowering unless they are grown for seed production, in which case precautions would usually be taken to avoid crossing with canola or Indian mustard (OGTR, 2017). Forage brassicas usually do not reach flowering due to re-sowing to new pastures or crops after grazing, and because flowering crops should not be fed to livestock (Harrington, 2012; Heritage Seeds, 2016).

99. Canola is widely grown as a commercial crop in Australia. Most of the canola crop is herbicide tolerant with one of three different herbicide tolerance traits (either GM or non-GM). These include non-GM canola varieties that are triazine tolerant (TT) and imidazolinone tolerant (IMI; Clearfield®), or GM glyphosate tolerant (GT; Roundup Ready® + TruFlex™ Roundup Ready®), and stacked varieties with tolerance to two herbicides (TT + IMI, TT + GT, IMI + GT) (Shackley et al., 2019; Matthews et al., 2020). One non-GM herbicide tolerance trait, Clearfield®, is also commercially available in juncea canola (GRDC, 2017). The majority of canola varieties are hybrids, with only a few non-GM canola varieties (including TT canola), and juncea canola available as open pollinated varieties (Shackley et al., 2019; Matthews et al., 2020).

100. No GM juncea canola has been approved for commercial cultivation in Australia to date, although a number of GM canola varieties have been, as listed in Table 7. MON 88302 (TruFlex™ Roundup Ready® canola), as a newer variant of Roundup Ready® canola, has been available to growers since 2019 (Shackley et al., 2019; Matthews et al., 2020) and commercial cultivation commenced from 2019 (information provided by Bayer under the DIR 127 licence). Although GM glufosinate tolerant varieties have been approved by the Regulator since 2003, the LibertyLink® trait (glufosinate tolerance) is only expected to be grown in demonstration trials in 2022 before becoming available to Australian growers in future (BASF website, accessed March 2022). MS8 × RF3 × MON 88302 (InVigor® x TruFlex™ Roundup Ready® canola) and MS11 × RF3 × MON 88302 canola with dual glufosinate and glyphosate tolerance and the Optimum™ GLY canola with glyphosate tolerance have also been approved by the Regulator for commercial cultivation since 2016, but they have only been grown on small scales in various States in Australia to date (information provided by the relevant licence holders).

Table 7 GM canola approved for commercial cultivation in Australia

DIR licence	Year approved	Year of first commercial cultivation	Trade name	GM traits
020/2002	2003	2008	Roundup Ready® Canola	Tolerance to glyphosate
021/2002	2003	-	InVigor® Canola	Tolerance to glufosinate; HBS
108	2011	-	InVigor® x Roundup Ready® Canola	Tolerance to glufosinate and glyphosate; HBS
127	2014	2019	TruFlex™ Roundup Ready® Canola	Tolerance to glyphosate
138	2016	-	InVigor® x TruFlex™ Roundup Ready® Canola	Tolerance to glufosinate and glyphosate; HBS
139	2016	-	Optimum™ GLY Canola	Tolerance to glyphosate
155	2018	-	N/A	Tolerance to glufosinate; omega-3 oil content
175	2021	-	N/A	Tolerance to glufosinate; HBS
178	2021	-	N/A	Tolerance to glufosinate and glyphosate; HBS

101. Significant pre-and post-fertilisation 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, 2012). It is considered that, if such hybrids were to be produced 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 *S. arvensis* was low to negligible (Warwick and Martin, 2013). More detailed discussion of *B. juncea* hybridisation can be found in the biology document (OGTR, 2017).

7.3.2 Presence of related native plants in the receiving environment

102. Members of the Brassicaceae family form part of the indigenous flora in regions throughout Australia. Widespread genera of Australian Brassicaceae include *Arabidella*, *Blennodia*, *Cuphonotus*, *Geococcus*, *Harmsiodoxa*, *Menkea*, *Microlepidium*, *Phlegmatospermum*, and *Stenopetalum* (tribe Microlepidieae); *Barbarea*, *Cardamine* and *Rorippa* (tribe Cardamineae); and *Lepidium* (tribe Lepideae) (Western Australian Herbarium, 1998–; Heenan et al., 2012; OGTR, 2017; de Salas and Baker, 2018; CANBR, 2019; Edginton, 2019).

103. Gene flow is less likely to occur between more distantly related species. It is not plausible that gene flow would occur from *B. juncea* to any native Australian plants, which belong to less closely related genera, under natural conditions (OGTR, 2017).

7.3.3 Presence of other biotic factors

104. A number of diseases have the potential to reduce the yield of juncea canola. Blackleg disease caused by the fungal pathogen *Leptosphaeria maculans* is the most serious disease affecting commercial canola production in Australia (OGTR, 2017). Juncea canola can be infected with the blackleg fungus. However, if juncea canola is grown in the low rainfall zone, blackleg is considered a lesser problem (GRDC, 2009; Haskins et al., 2009). Other damaging diseases that can infect juncea canola include fungal pathogens such as white rust (*Albugo candida*), stem rot (*Sclerotinia sclerotiorum*), clubroot (*Plasmodiophora brassicae*) and damping-off (*Rhizoctonia solani*), and viruses such as beet western yellows virus, turnip mosaic virus and cauliflower mosaic virus (GRDC, 2009; Haskins et al., 2009).

105. Juncea canola is subject to the same range of pests as canola, but the range of pests and populations of specific pests may be different to those of canola grown in medium–high rainfall environments, as the crop is likely to be grown in low rainfall environments (Haskins et al., 2009). Like canola, juncea canola is most susceptible to insect pests during establishment of the crop, particularly from earth mites (*Halotydeus destructor*), blue oat mites (*Penthaleus major*, *P. falcatus*, and *P. tectus* sp. n.), lucerne fleas (*Sminthurus viridis*), cutworms (*Agrotis* spp.) and aphids (*Brevicoryne brassicae*, *Myzus persicae* and *Lipaphis pseudobrassicae*; as viral vectors). From flowering to crop maturity, severe damage can be caused by aphids, Rutherglen bugs (*Nysius vinitor*), diamondback moth caterpillars (*Plutella xylostella*) and heliothis caterpillars (*Helicoverpa armigera*).

106. Like canola, juncea canola is also highly susceptible to weed competition during the early stages of growth (GRDC, 2009). The most problematic weeds include grass weeds, such as rigid ryegrass (*Lolium rigidum*, annual ryegrass), vulpia and wild oat, volunteer cereals, and weeds from the *Brassicaceae* family, which can also reduce product quality through seed contamination (Sutherland, 1999). Common *Brassicaceae* weeds are wild radish (*R. raphinastrum*), Indian hedge mustard (*Sisymbrium orientale*), shepherd's purse (*Capsella bursa-pastoris*), wild turnip (*Brassica tournefortii*), turnip weed (*Rapistrum rugosum*), charlock (*Sinapis arvensis*), musk weed (*Myagrum perfoliatum*) and Buchan weed (*H. incana*) (Sutherland, 1999).

7.3.4 Weed resistance to glufosinate herbicides

107. There is potential for development of herbicide-resistant weeds if glufosinate herbicides are inappropriately used with RF3 juncea canola. The repetitive use of a single herbicide, or herbicide group[10], increases the likelihood of weeds with evolved genetic traits conferring herbicide resistance are able to persist (Busi et al., 2013). Integrated management practices help to avoid selection of herbicide resistant weeds (GRDC, 2019).

108. Herbicide resistance comes under the regulatory oversight of the APVMA. The APVMA has primary regulatory responsibility for agricultural chemicals in Australia and operates the national system that evaluates, registers and regulates agricultural and veterinary chemical products. Any changes to a product that is already on the market must also be referred to the APVMA.

109. Weeds resistant to glufosinate herbicides have been reported overseas; however, no glufosinate-resistant weed species have been reported in Australia (Heap, 2022). The species that are currently known to have developed resistance to glufosinate are annual bluegrass (*Poa annua*; USA), goosegrass (*Eleusine indica*; Malaysia), Italian ryegrass (*Lolium multiflorum*; NZ, USA), Palmer amaranth (*Amaranthus palmeri*; USA), perennial ryegrass (*L. perenne*; NZ) and rigid ryegrass (*L. rigidum*, annual ryegrass[11]; Greece).

110. Stewardship guides and CMPs are prepared by companies selling herbicide tolerant canola seed. These guides are to be followed when growing herbicide tolerant varieties to control canola volunteers, and prevent or delay the development of herbicide resistant weeds. The applicant states that they will provide farmers with a CMP for RF3 juncea canola, which is the same as that developed for RF3 canola (see Section 7.1).

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

[11] In Australia, the name 'annual ryegrass' may refer to either *Lolium rigidum* or *L. multiflorum.*

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

111. The introduced genes were originally isolated from naturally occurring organisms that are already prevalent in the environment.

112. The *bar* gene was isolated from the common bacterium *S. hygroscopicus*, which is a saprophytic, soil-borne microorganism that is not considered a pathogen of plants, humans or other animals (OECD, 1999). Genes encoding PAT and similar acetyltransferase enzymes are present in a range of common soil bacteria, and the encoded proteins are not known to be toxic or allergenic (Hérouet et al., 2005).

113. The bacterium *B. amyloliquefaciens*, from which the *barstar* gene was obtained, is a commonly occurring soil bacterium that is widespread in nature and is frequently used in industry. Production of 11 food-grade enzymes by *B. amyloliquefaciens* has been assessed as safe by FSANZ (*Australia New Zealand Food Standards Code* – Schedule 18, accessed October 2020). An assessment of *B. amyloliquefaciens* by Environment Canada and Health Canada (2015) did not identify adverse effects to human health or towards aquatic or terrestrial plants, vertebrates or invertebrates in a variety of environments.

114. Barstar is a ribonuclease inhibitor protein produced by *B. amyloliquefaciens* and specifically inhibits the ribonuclease enzyme Barnase function. Nuclease enzymes and inhibitor proteins are ubiquitous in nature and can be found in plants, animals and microorganisms. Antibacterial effector/immunity systems similar to Barnase/Barstar are widespread in bacteria (Benz and Meinhart, 2014). Therefore, both the source organism (*B. amyloliquefaciens*) and the ribonuclease inhibitor protein encoded by the introduced gene would be commonly encountered by other organisms in the environment.

115. Short regulatory sequences are derived from the bacterium *Agrobacterium tumefaciens,* the plants *Arabidopsis thaliana* (thale cress) and *Nicotiana tabacum* (tobacco). Although *A. tumefaciens*, is a plant pathogen, and tobacco produces toxins and carcinogens, the regulatory sequences comprise a small part of their total genome, and in themselves have no pathogenic, toxic or carcinogenic properties. With the exception of tobacco, which is no longer grown commercially in Australia, all the source organisms for the introduced genetic elements are widespread and prevalent in the Australian environment and thus humans and other organisms would commonly encounter their genes, encoded proteins and regulatory sequences.

Chapter 2 Risk assessment

Section 1 Introduction

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

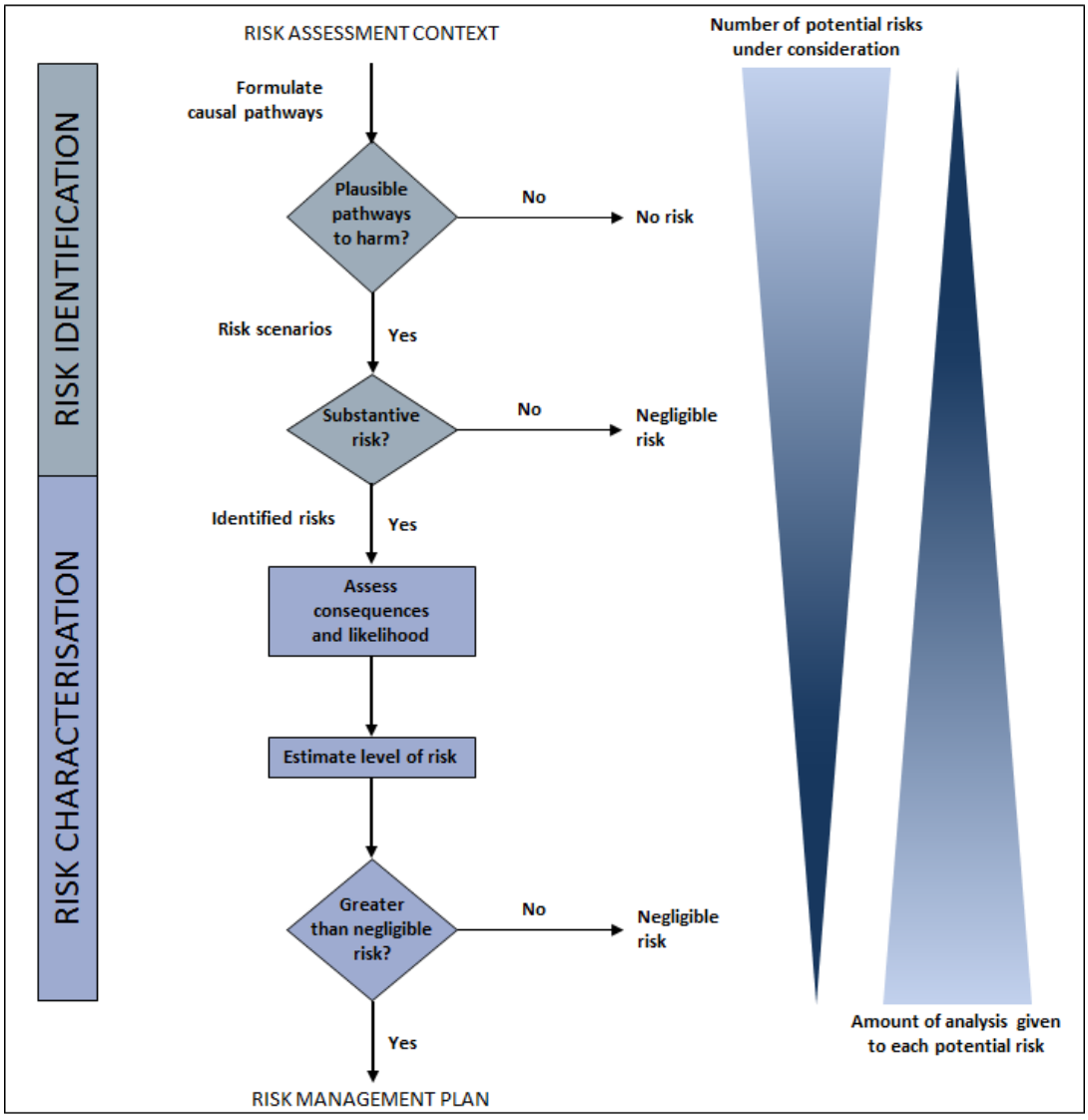

Figure 2 The risk assessment process

117. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, previous agency experience, reported international experience and consultation (OGTR, 2013).

118. Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are called risk scenarios.

119. Risk scenarios are screened to identify substantive risks, which are risk scenarios that are considered to have some reasonable chance of causing harm. Risk scenarios that could not plausibly occur, or do not lead to harm in the short and long term, do not advance in the risk assessment process (Figure 2), i.e. the risk is considered no greater than negligible.

120. Risk scenarios identified as substantive risks are further characterised in terms of the potential seriousness of harm (consequence assessment) and the likelihood of harm (likelihood assessment). The consequence and likelihood assessments are combined to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

121. 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 considered in postulating risk scenarios (Keese et al., 2014). Risk scenarios postulated in previous RARMPs prepared for licence applications for the same or similar GMOs are also considered.

Section 2 Risk identification

122. Postulated risk scenarios are comprised of three components (Figure 3):

i. The source of potential harm (risk source),

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

iii. Potential harm to people or the environment.

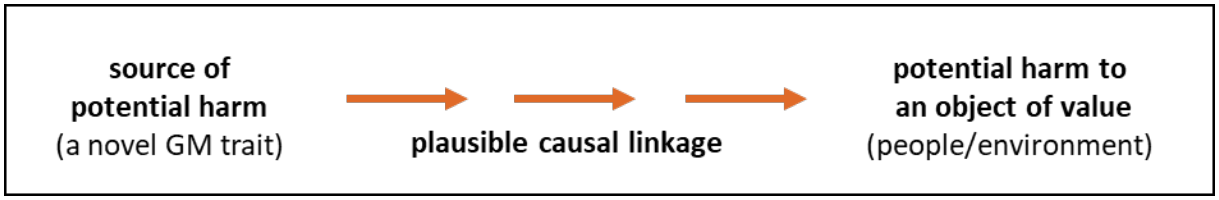

Figure 3 Components of a risk scenario

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

- the proposed dealings,
- any proposed limits including the extent and scale of the proposed dealings,
- any proposed controls to limit the spread and persistence of the GMO, and
- the characteristics of the parent organism(s).

2.1 Risk source

124. 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.

125. As discussed in Chapter 1, Section 6.1, the RF3 juncea canola proposed for release is the result of conventional breeding between RF3 canola and a non-GM juncea canola line. RF3 juncea canola has been modified by the introduction of a gene for tolerance to the herbicide glufosinate and a gene for male fertility restoration in a HBS. The introduced genes and their encoded proteins are considered further as potential sources of risk.

126. The introduced genes are controlled by introduced regulatory sequences. These regulatory sequences are derived from common plants and a common soil bacterium (Table 4). 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). As described in Chapter 1, these sequences have been widely used in other GMOs, including in GM canola grown commercially in Australia and overseas, without reports of adverse effects. Hence, potential for harm from the regulatory elements will not be considered further.

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

2.2 Causal pathway

128. The following factors are considered when postulating plausible causal pathways to potential harm:

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

129. Although all of these factors are taken into account, some are not included in risk scenarios because they are regulated by other agencies, have been considered in previous RARMPs, or are not expected to give rise to substantive risks (see Sections 2.2.1 to 2.2.5 below).

2.2.1 Tolerance to abiotic factors

130. The geographic range of non-GM juncea canola in Australia is limited by a number of abiotic factors including climate and soil compatibility, as well as water and nutrient availability (OGTR, 2017). The introduced genes are unlikely to make RF3 juncea canola plants more tolerant to abiotic stresses that are encountered in the environment and are therefore unlikely to alter the potential distribution of RF3 juncea canola plants. Also, as discussed in Chapter 1, Section 6.2.3, the response of RF3 juncea canola to abiotic factors is considered to be equivalent to the non-GM counterpart. Therefore, tolerance to abiotic stresses will not be considered further.

2.2.2 Development of herbicide resistant weeds through selective pressure

131. There is some potential for development of herbicide resistant weeds if a herbicide tolerant juncea canola and its corresponding herbicide are used inappropriately. The repetitious use of a single herbicide, or herbicide group, increases the likelihood of selecting weeds that have developed herbicide resistance through natural mechanisms (Gressel, 2002). This is not a novel issue associated only with GMOs, as most canola currently grown in Australia is herbicide tolerant, by either non-GM or GM mechanisms (Chapter 1, Section 7.3.1).

132. The genetic modification to the RF3 juncea canola proposed for release confers tolerance to glufosinate herbicides. Six glufosinate-resistant weed species have been identified overseas but none have been reported in Australia (Chapter 1, Section 7.3.4).

133. Development of herbicide resistant weeds through selective pressure comes under the regulatory oversight of the APVMA, which has primary regulatory responsibility for agricultural chemicals in Australia. The APVMA assesses all herbicides used in Australia and sets their conditions of use. Where the use pattern of a chemical product changes in association with a genetically modified crop plant, the APVMA will assess the new use pattern of the chemical. Therefore, the issue of development of herbicide resistant weeds through selective pressure will not be further considered in this risk assessment. The development of herbicide tolerant weeds through gene transfer will be considered below.

2.2.3 Herbicide metabolites

134. The potential toxicity of a herbicide is not in scope of this assessment as the herbicide is not part of the genetic modification. Potential toxicity of the metabolites of glufosinate herbicide is discussed in Chapter 1, Section 5.1.4.

135. If the GM herbicide tolerant canola lines are to be commercially cultivated in Australia, the potential toxicity of glufosinate herbicide and its metabolites is considered by the APVMA in its assessment of a new use pattern for registration. Ultimately, the APVMA has regulatory responsibility for the supply of agricultural chemicals, including herbicide products, in Australia. Therefore, the potential toxicity of glufosinate herbicide and its metabolites will not be further considered.

2.2.4 Horizontal gene transfer

136. The potential for horizontal gene transfer (HGT) and any possible adverse outcomes has been reviewed in the literature (Keese, 2008) and assessed in previous RARMPs. No risk greater than negligible was identified, due to the rarity of HGT events and because the gene sequences (or sequences which are homologous to those in the current application) are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, HGT will not be assessed further.

2.2.5 Unauthorised activities

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

2.3 Potential harm

138. Potential harms from GM plants include:

- harm to the health of people or desirable organisms, including toxicity/allergenicity
- reduced biodiversity for nature conservation

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

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

2.4 Postulated risk scenarios

140. Five risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 8 and discussed in depth in Sections 2.4.1 to 2.4.5. Postulation of risk scenarios considers impacts of RF3 juncea canola or its products on people undertaking the dealings, as well as impacts on people and the environment exposed to RF3 juncea canola or its products as the result of commercial use or the spread and persistence of plant material.

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

Table 8 Summary of risk scenarios from the proposed dealings

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced genes for glufosinate tolerance and male fertility restoration	Commercial cultivation of GM juncea canola expressing the introduced genes ⬇ Exposure of people and other organisms via contact or consumption of GM juncea canola plants or products	• Increased toxicity or allergenicity for people, or • Increased toxicity for other desirable organisms	No	• The introduced proteins are not considered toxic or allergenic to people and other desirable organisms. • The parental GM canola line with the introduced genes has a history of safe use. • The introduced genes and proteins are widespread in the environment. • The GM juncea canola seed is compositionally equivalent to non-GM juncea canola seed.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
2	Introduced genes for glufosinate tolerance and male fertility restoration	Commercial cultivation of GM juncea canola expressing the introduced genes ⬇ Establishment of volunteer GM juncea canola plants in agricultural areas ⬇ Reduced effectiveness of weed management measures to control volunteer GM juncea canola plants	• Reduced establishment or yield of desirable agricultural crops, or • Increased reservoir for pests or pathogens	No	• The genetic modification only gives an advantage to the GM juncea canola plants in managed environments, where glufosinate herbicide is applied. • The GM juncea canola can be controlled using integrated weed management, including use of other classes of herbicide.
3	Introduced gene for glufosinate tolerance and male fertility restoration	Commercial cultivation of GM juncea canola expressing the introduced genes ⬇ Dispersal of GM juncea canola seed to nature reserves or intensive use areas ⬇ Establishment of GM plants in intensive use areas or nature reserves ⬇ Reduced effectiveness of weed management measures to control feral GM plants	• Reduced establishment of desirable native vegetation, or • Reduced services from the land use	No	• The GM and non-GM juncea canola have similar intrinsic characteristics for spread and persistence. • The GM juncea canola is susceptible to the biotic and abiotic stresses that normally restrict the geographic range and persistence of juncea canola. • Weed management strategies other than glufosinate can be used to control feral GM juncea canola.
4	Introduced genes for glufosinate tolerance and male fertility restoration	Commercial cultivation of GM juncea canola in agricultural areas ⬇ Cross-pollination with other Indian mustard, including Indian mustard with other herbicide tolerance traits ⬇ Establishment of hybrid GM Indian mustard plants expressing the herbicide tolerance gene as volunteers ⬇ Reduced effectiveness of weed management measures to control the hybrid plants	• Reduced establishment or yield of desirable agricultural crops, or • Increased reservoir for pests and pathogens	No	• Hybrids between the GMO and other Indian mustard would be generated at low levels. • Multiple-herbicide tolerant hybrids can be controlled using integrated weed management.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
5	Introduced genes for glufosinate tolerance and male fertility restoration	Commercial cultivation of GM juncea canola in agricultural areas ⬇ Cross-pollination with sexually compatible Brassica crops or weeds ⬇ Establishment of hybrid GM Brassica plants expressing the introduced genes as volunteers, or Introgression of the introduced genes into weeds ⬇ Establishment of hybrids expressing the herbicide tolerance gene ⬇ Reduced effectiveness of weed management measures to control weeds expressing the introduced genes	• Reduced establishment or yield of desirable agricultural crops	No	• Hybrids between the GM juncea canola and Brassica crop or weed species would occur at low or very low levels. • Hybrids can be controlled using integrated weed management. • It is highly unlikely that a GM herbicide tolerance gene would introgress into a Brassicaceae weed species.

2.4.1 Risk scenario 1

Risk source	Introduced genes for glufosinate tolerance and male fertility restoration
Causal pathway	⬇ Commercial cultivation of GM juncea canola expressing the introduced genes ⬇ Exposure of people and other organisms via contact or consumption of GM juncea canola plants or products ⬇
Potential harm	Increased toxicity or allergenicity for people OR Increased toxicity for other desirable organisms

Risk source

142. The source of potential harm for this postulated risk scenario is the introduced genes for glufosinate tolerance and male fertility restoration.

Causal pathway

143. The applicant proposes that RF3 juncea canola would be cultivated on a commercial scale in all suitable Australian agricultural cropping areas. The glufosinate tolerance gene *bar* is expressed in all parts of the RF3 juncea canola plant at all developmental stages including leaf, stem, root and seed. Expression of the *barstar* gene is restricted to the anthers (Chapter 1, Section 6.2.2).

144. RF3 juncea canola would enter general commerce and be used in the same ways as non-GM juncea canola and other canola. The general public could be exposed to oil from RF3 juncea canola, which would be sold for human consumption. However, refined canola oil is unlikely to contain any protein (FSANZ, 2017) and refined RF3 juncea canola oil would be no different from other canola oil.

145. People could be exposed to wind-borne GM juncea canola pollen by inhalation. The vast majority of wind-dispersed canola pollen travels less than 10 m from the pollen source (Hüsken and Dietz-Pfeilstetter, 2007) and the juncea canola pollen would be expected to have very similar behaviour, so this route of exposure would mainly apply to people who enter or pass close to GM juncea canola fields during flowering.

146. People involved in cultivating or processing RF3 juncea canola or using the GM juncea canola meal as animal feed, could be exposed to plant parts or products through contact.

147. Livestock could be exposed when consuming RF3 juncea canola as forage, whole seed or seed meal.

148. Wild animals, including birds, could enter juncea canola fields and feed on GM juncea canola seed or other plant parts. Pollinators such as bees would be exposed to nectar and pollen from the GM juncea canola. Soil organisms, such as earthworms, would contact root exudates or decomposing plant material after harvest. Therefore, these desirable organisms would be exposed to the GM juncea canola and plant material derived from them.

Potential harm

149. Toxicity is the adverse effect of exposure to a substance (Klaassen and Watkins, 2010). The effect of a toxic agent depends on the dose, duration of exposure and exposure route, e.g. inhalation, ingestion or via the skin. Responses may be either immediate or delayed. Allergic reactions are a type of adverse effect, resulting from sensitisation to a chemical, followed by an allergic response upon subsequent exposure (Klaassen and Watkins, 2010). Allergenicity is the potential for a chemical to be recognised by the body as a foreign substance and to elicit a (disproportionate) immunological reaction.

150. The *bar* and *barstar* genes introduced into RF3 juncea canola encode proteins that are well characterised. Based on all available information, these proteins are not known to be toxic or allergenic to humans, do not share relevant sequence homology with known toxins or allergens (Chapter 1, Section 5.1.3), and do not change the biochemical composition of the GM juncea canola seed (Chapter 1, Section 6.2.4).

151. FSANZ has determined that food derived from the parental RF3 canola is as safe for human consumption as food derived from conventional (non-GM) canola varieties (Chapter 1, Section 5.2). This approval also covers RF3 juncea canola (Chapter 1, Section 3.2). RF3 canola has also been approved for food and/or feed use in other countries (Chapter 1, Section 3.3). In addition, compositional analysis of seed from RF3 juncea canola confirmed that seed from this GM juncea canola is compositionally equivalent to seed from non-GM juncea canola varieties (Chapter 1, Section 6.2.4).

152. There have been no reported adverse effects on human or animal health from RF3 canola (Chapter 1, Section 3.1.2) or other commercial GM crops with the same introduced *bar* and/or *barstar* genes (Chapter 1, Section 5.1.3).

153. The introduced genes were isolated from common soil bacteria (Chapter 1, Section 7.4). Thus, it is expected that desirable soil organisms are regularly exposed to the introduced proteins or their degradation products.

Conclusion

154. Risk scenario 1 is not identified as a substantive risk because the expressed proteins are not considered toxic or allergenic to people, GM canola lines containing the introduced genes have a history of safe use in Australia and overseas, the introduced genes and proteins are widespread in the environment and the GM juncea canola seed is compositionally equivalent to non-GM juncea canola seed. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk source	Introduced genes for glufosinate tolerance and male fertility restoration
Causal pathway	⬇ Commercial cultivation of GM juncea canola expressing the introduced genes ⬇ Establishment of volunteer GM juncea canola plants in agricultural areas ⬇ Reduced effectiveness of weed management measures to control volunteer GM juncea canola plants ⬇
Potential harm	Reduced establishment or yield of desirable agricultural crops OR Increased reservoir for pests or pathogens

Risk source

155. The source of potential harm for this postulated risk scenario is the introduced genes for glufosinate tolerance and male fertility restoration.

Causal pathway

156. The applicant proposes that RF3 juncea canola would be cultivated on a commercial scale. In current Australian agriculture, canola volunteers requiring weed management are likely to be found in fields for up to three years after growing a canola crop (Australian Oilseeds Federation, 2019). Although information regarding *B. juncea* seed germination and dormancy is limited (OGTR, 2017), the seed germination and cold tolerance tests carried out in controlled plant growth chambers showed that over 99% RF3 juncea canola and control seeds germinated at normal temperature range (5 - 35°C) but only about a quarter of seeds germinated after cold treatment at -5 ± 5°C for 10 days (Chapter 1, Section 6.2.3). This indicates that seeds from RF3 juncea canola and its parental non-GM juncea canola do not display primary dormancy. However, secondary dormancy may be induced when seeds are buried in soil or stored under dry and cold conditions (OGTR, 2017). As juncea canola will most likely be grown in low rainfall areas in Australia, volunteers requiring weed management are likely to be found in fields for a longer time than canola after growing a juncea canola crop. Field trials in the USA and Canada indicated no biologically meaningful differences between RF3 juncea canola and non-GM juncea canola with respect to the characteristics contributing to spread and persistence (e.g. seed production, pod shattering and responses to environmental stressors; Chapter 1, Section 6.2.3); it is expected to produce similar numbers of volunteers as non-GM juncea canola.

157. Volunteer juncea canola plants are likely to occur following dispersal of GM juncea canola seeds within agricultural areas (Chapter 1, Section 4.2). Short-range dispersal of juncea canola seed into field margins or adjacent fields could occur via pod shattering or transport of juncea canola plant material from windrows by strong winds (OGTR, 2017), although windrowing is not a standard operation for juncea canola (Chapter 1, Section 7.1). Short to medium-range dispersal of juncea canola seed within agricultural areas could be mediated by human activities such as movement of agricultural machinery used during juncea canola sowing or harvest. Dispersal of viable juncea canola seed by animals, including birds, via consumption and excretion is also possible at very low levels (OGTR, 2017).

158. RF3 juncea canola only has a survival advantage in the presence of glufosinate, noting that the introduced gene to restore male fertility does not influence characteristics in the GM juncea canola for weediness, including tolerance to herbicides. However, glufosinate is not widely used in broadacre cropping or management along roadsides (NSW DPI, 2018). Glufosinate herbicides are not effective in controlling juncea canola volunteers in situations where RF3 juncea canola had been grown previously. BASF will develop a CMP for RF3 juncea canola to be followed when growing RF3 juncea canola (see Chapter 1, Sections 7.1 and 7.3.4).

159. All herbicides sold in Australia must be labelled with their mode of action for the purpose of resistance management (APVMA website, accessed March 2022). The mode of action is indicated by a letter code on the product label. Herbicides belonging to nine mode of action groups are available for juncea canola volunteer control in Australia (Chapter 1, Section 4.2.4). Herbicides from different mode of action groups (B, C, F, G, H, I, L, M and Q) or products with multiple mode of action groups (B+G, B+I, C+F, C+H, C+I, F+I, G+I, G+M, H+I, L+Q and C+F+I) could be used to control canola volunteers in various crop and non-crop situations, which also apply to juncea canola volunteer control (Australian Oilseeds Federation, 2019). Further details of registered herbicide products are available on the APVMA PubCRIS database.

160. RF3 juncea canola volunteers only have a survival advantage over non-GM juncea canola volunteers in the presence of glufosinate herbicides. They are as susceptible as non-GM juncea canola to all herbicides other than glufosinate herbicides. RF3 juncea canola volunteers could, therefore, be controlled using integrated weed management practices, which include using a variety of other herbicides assessed and approved by the APVMA, as well as non-chemical management methods currently used to control non-GM canola, such as mowing, grazing or cultivation (Australian Oilseeds Federation, 2019).

Potential harm

161. Volunteer *B. juncea* is a weed of agricultural production systems (Groves et al., 2003). If left uncontrolled, volunteer RF3 juncea canola plants are expected to establish and compete with other crops in the same way as non-GM juncea canola. However, as discussed above, there are alternative methods to control the GM volunteers and therefore the number of volunteers persisting in agricultural areas would be similar to non-GM juncea canola volunteers. Therefore, RF3 juncea canola volunteers that are effectively controlled would not be expected to cause greater yield reduction of desired crops than non-GM *B. juncea* volunteers that are effectively controlled. Also, the RF3 juncea canola has no advantage over non-GM *B. juncea* with respect to abiotic stressors other than glufosinate herbicide (Chapter 1, Section 6.2.3).

162. Like canola, juncea canola crops are susceptible to a range of pests and diseases (Chapter 1, Section 7.3.3). Volunteer juncea canola can act as a reservoir for juncea canola and canola (*B. napus*) pests and pathogens. For example, volunteer juncea canola plants can be a source of diamondback moth infestation and can act as a reservoir for viral and fungal pathogens of canola, such as clubroot (GRDC, 2009). Field trials of RF3 juncea canola did not reveal any significant differences between RF3 juncea canola and non-GM *B. juncea* varieties for disease stress or insect stress ratings in Canada and the USA (Chapter 1, Section 6.2.3). Effective control of juncea canola volunteers (both GM and non-GM) reduces the potential for volunteers to act as reservoirs for pests and diseases.

Conclusion

163. Risk scenario 2 is not identified as a substantive risk because the genetic modification only gives an advantage to the GM juncea canola plants in managed environments where glufosinate herbicides is applied, and because the GM juncea canola can be controlled using integrated weed management. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk scenario 3

Risk source	Introduced genes for herbicide tolerance and male fertility restoration
Causal pathway	⬇ Commercial cultivation of GM juncea canola expressing the introduced genes ⬇ Dispersal of GM juncea canola seed to intensive use areas or nature reserves ⬇ Establishment of GM plants in intensive use areas or nature reserves ⬇ Reduced effectiveness of weed management measures to control feral GM plants ⬇
Potential harm	Reduced establishment of desirable native vegetation OR Reduced services from the land use

Risk source

164. The source of potential harm for this postulated risk scenario is the introduced genes for glufosinate tolerance and male fertility restoration.

Causal pathway

165. The applicant proposes to grow RF3 juncea canola on a commercial scale. After harvest, the GM juncea canola seed would be transported for processing or storage. Seed spillages could lead to the establishment of feral canola populations in intensive use areas, e.g. along transport routes, or near processing, feeding or storage sites.

166. If transport routes passed through or were near nature reserves, dispersal of GM juncea canola seeds into nature reserves could occur via spillages. Spillage of canola seed has been observed along roadsides (Bailleul et al., 2012) and it is assumed that this could also happen with transport of GM juncea canola seed. If this does occur, there is potential for GM juncea canola to establish as feral populations and become a source for further spread into nature reserves. However, as discussed in Chapter 1, Section 4.2.3, although feral *B. juncea* plants are often observed growing on roadsides or railway easements in Australia, these populations are thought to be reliant on re-supply of seed from spillages, rather than forming self-sustaining weed populations.

167. Whole seeds could be used as livestock feed and feral GM juncea canola could establish in and around animal feeding areas.

168. Like canola, juncea canola seed is small and easily dispersed. Although juncea canola is less prone to pod shatter than canola, juncea canola pods can still shatter some seeds prior to and during harvest, allowing for the establishment of volunteers in areas near the cultivated field similar to canola (Ellstrand, 2018). Dispersal of viable juncea canola seed into nature reserves by animals, including birds, via consumption and excretion is possible at very low levels (OGTR, 2017). Viable seeds could also be dispersed into intensive use areas or nature reserves via extreme weather, such as flooding or high winds (OGTR, 2017).

169. If seed from the GM juncea canola dispersed into intensive use areas or nature reserves, the seeds could germinate and establish a population of GM plants. The GM juncea canola proposed for release is similar to non-GM juncea canola with respect to most of the intrinsic characteristics contributing to spread and persistence, such as germination and establishment, seedling vigour, seed production and pod shattering (Chapter 1, Section 6.2.3). Therefore, the level of volunteers is expected to be similar to non-GM juncea canola. The genetic modification is also not expected to alter the tolerance of GM plants to abiotic or biotic stresses that normally restrict the geographic range and persistence of juncea canola (Chapter 1, Sections 7.2 and 7.3). Nor is it likely to be more resistant to

biotic stresses – pests and pathogens (Chapter 1, Section 7.3.3). Therefore, feral GM juncea canola would have no survival advantage over non-GM juncea canola in the absence of glufosinate herbicide and is not expected to be more persistent than non-GM juncea canola.

170. The trait of glufosinate tolerance could affect a GM plant's tolerance to weed management practices in areas where glufosinate herbicides are used. The main herbicide used for roadside weed management in Australia is glyphosate (Storrie, 2018).

171. In nature reserves where glufosinate is not used for weed control, the GM juncea canola is not expected to have any survival advantage over non-GM juncea canola. In a study observing survival of glyphosate tolerant GM canola seeds dispersed into two natural areas, the population became extinct in either 0 or 3 years. The authors concluded that the GM trait did not confer a fitness advantage that would result in greater establishment and persistence (Busi and Powles, 2016). There is no evidence that the introduced traits in RF3 juncea canola provides a fitness advantage compared to non-GM parental juncea canola. Thus, considering that juncea canola is not a persistent weed in natural undisturbed habitats in Australia (Chapter 1, Section 4.2.3), it is also highly unlikely that the GM juncea canola with glufosinate tolerance and male fertility restoration would persist in such areas.

Potential harm

172. If the GM juncea canola expressing the introduced genes were able to establish and persist in nature reserves, this could reduce the establishment of desirable native vegetation. It could give rise to lower abundance of desirable species, reduced species richness, or undesirable changes to species composition. Feral juncea canola could also potentially reduce services from the land use by decreasing the amenity of nature reserves for nature-based tourism.

173. Juncea canola can grow to a height of over 2 m along roadsides (OGTR, 2017) and is highly visible when in flower. Feral juncea canola on roadsides or along railway lines could reduce services from the land use by obstructing lines of sight around corners or to signs if present in large numbers. While the Western Australian Department of Biodiversity, Conservation and Attractions listed feral canola as one of 60 weeds that threaten rail and roadside vegetation by lowering the biodiversity and aesthetic value of the verge, and recommends that management of these weeds be a priority along roads of high conservation value (Roadside Conservation Committee, 2017), it did not list feral *B. juncea* as a concern. Also, *B. juncea* is not listed as an established weed causing adverse agricultural, social or environmental impacts by weed managers around Australia in the latest national weeds data collection survey conducted by Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) (Ng et al., 2021), indicating that feral *B. juncea* was not a weed of national concern.

174. None of these potential harms are increased in the GM juncea canola proposed for release compared to non-GM juncea canola and the GM parental canola lines.

Conclusion

175. Risk scenario 3 is not identified as a substantive risk because the GM juncea canola is similar to non-GM juncea canola with respect to the characteristics contributing to spread and persistence, it is susceptible to the abiotic or biotic stresses that normally restrict the geographic range and persistence of juncea canola and can be controlled using weed management strategies other than glufosinate use. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.4 Risk scenario 4

Risk source	Introduced genes for herbicide tolerance and male fertility restoration
Causal pathway	⬇ Commercial cultivation of GM juncea canola in agricultural areas ⬇ Cross-pollination with other Indian mustard, including Indian mustard with other herbicide tolerance traits ⬇ Establishment of hybrid GM plants expressing the introduced genes as volunteers ⬇ Reduced effectiveness of weed management measures to control the hybrid plants ⬇
Potential harm	Reduced establishment or yield of desirable agricultural crops OR Increased reservoir for pests or pathogens

Risk source

176. The source of potential harm for this postulated risk scenario is the introduced genes for glufosinate tolerance and male fertility restoration.

Causal pathway

177. The applicant proposes that RF3 juncea canola would be cultivated on a commercial scale in all suitable agricultural cropping areas of Australia. Cross pollination between the GM juncea canola proposed for release and other Indian mustard would most likely occur when different Indian mustard crops are grown in adjacent paddocks and flower synchronously. Cross pollination may also occur at a smaller scale with volunteer or feral Indian mustard populations.

178. Outcrossing rates between neighbouring commercial canola fields in Australia are less than 0.1% averaged over whole fields (Rieger et al., 2002). Although no information is available regarding *B. juncea* pollen movement in Australia, it is considered to be very similar to that observed for canola (OGTR, 2017). However, this is an area of uncertainty for this risk assessment.

179. No GM Indian mustard with a herbicide tolerance trait has been authorised for commercial cultivation in Australia. The only available herbicide tolerant Indian mustard variety is the non-GM Clearfield® juncea canola (Chapter 1, Section 7.3.1). Crossing may occur between the GM juncea canola and Clearfield® juncea canola to produce a hybrid with tolerance to both glufosinate and imidazolinone herbicides. However, the current scale of Indian mustard production in Australia is very small (Chapter 1, Section 4.1). Correspondingly, the likelihood of both GM and non-GM (either herbicide tolerant or not) crops flowering concurrently in the same area (such that hybridisation was possible), in conjunction with the low likelihood of hybridisation between the two crops, means that few hybrids would be expected between the GM juncea canola and other Indian mustard in each growing season.

180. Hybrid seed with the GM trait could disperse within agricultural areas, to intensive use areas, or to nature reserves, by the same mechanisms described in Risk Scenarios 2 and 3. Volunteer or feral progeny of these hybrid plants could germinate and grow in these areas. In addition, if a field that is adjacent to the GM juncea canola is planted with an open pollinating Indian mustard variety, the farmer may retain seed, including a proportion of GM hybrid seed, for future planting.

181. Crossing between the GM juncea canola and non-GM, non-herbicide tolerant Indian mustard varieties would result in hybrid plants highly similar to the GM juncea canola proposed for release. Therefore, the progeny is not expected to pose any greater risks than the GM juncea canola proposed for release.

Potential harm

182. If left uncontrolled in agricultural areas, volunteer hybrid plants could establish and compete with other crops. In addition, surviving volunteer hybrids could act as a reservoir for juncea canola or canola pests or pathogens, as described in Risk scenario 2. As a result, the establishment and yield of desirable agricultural crops might be reduced.

183. However, additional herbicide tolerance traits are not expected to provide a survival advantage to the GM hybrids, except in the presence of the herbicides to which they are tolerant. Hybrid volunteers that are tolerant to both glufosinate and imidazolinone herbicides could be controlled by herbicides belonging to other mode of action groups, or by non-chemical management practices, as discussed in Risk scenario 2. Individuals tolerant to more than one herbicide are as susceptible to alternative herbicides as single-herbicide tolerant canola plants or their non-GM counterparts. The introduced male fertility restorer does not contribute herbicide tolerance.

184. In addition, the applicant will have a CMP ready that RF3 juncea canola growers are required to follow when growing the GM juncea canola (Chapter 1, Sections 7.1 and 7.3.4). This includes management strategies that aim to control juncea canola volunteers, minimise gene flow, and prevent or delay the development of herbicide resistant weeds.

185. In summary, considering the low likelihood of hybrid plants occurring and the management options available to control any hybrids, if those management practices are used effectively, hybrid volunteers are expected to be present at low densities and no greater numbers than for non-GM juncea canola. Small numbers of volunteers would have limited capacity to cause adverse effects.

Conclusion

186. Risk scenario 4 is not identified as a substantive risk because hybrids between the GM juncea canola and other Indian mustard and canola would be generated at a low level, and because any double-herbicide tolerant hybrids can be controlled using integrated weed management. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.5 Risk scenario 5

Risk source	Introduced genes for glufosinate tolerance and male fertility restoration
Causal pathway	⬇ Commercial cultivation of GM juncea canola in agricultural areas ⬇ Cross-pollination with sexually compatible Brassica crops or weeds ⬇ Establishment of hybrid GM Brassica plants expressing the introduced genes as volunteers, or Introgression of the introduced genes into weeds ⬇ Establishment of weeds expressing the introduced genes ⬇ Reduced effectiveness of weed management measures to control weeds expressing the introduced genes ⬇
Potential harm	Reduced establishment or yield of desirable agricultural crops

Risk source

187. The source of potential harm for this postulated risk scenario is the introduced genes for herbicide tolerance and male fertility restoration.

Causal pathway

188. The applicant proposes that the GM juncea canola be cultivated on a commercial scale in all agricultural cropping areas. This could bring it into proximity to other Brassica crop species, such as canola, vegetables and forage crops, as well as sexually compatible weed species.

Interactions with Brassica crop species

189. Pollen flow between the GM juncea canola proposed for release and other Brassica crop species could occur if the Brassica crops were grown near the GM juncea canola and flowered synchronously. Canola crops could readily cross-pollinate with the GM juncea canola. Brassica vegetable crops are generally harvested prior to flowering unless they are grown for seed production, in which case precautions would usually be taken to avoid crossing of the seed crops with oilseed Indian mustard and canola (Chapter 1, Section 7.3.1). Brassica forage crops usually do not reach flowering due to heavy grazing. Cross-pollination could also occur with Brassica volunteers.

190. Hybrids between *B. juncea* and *B. napus* have been observed in the field, are fertile, and often have high fitness (Liu et al., 2010). Crossing between the GM juncea canola and non-GM, non-herbicide tolerant canola varieties would result in hybrid plants highly similar to the GM juncea canola proposed for release. Therefore, the progeny would not be expected to pose any greater risk than the GM juncea canola proposed for release.

191. Crossing may also occur between the GM juncea canola and non-GM and GM herbicide tolerant canola varieties. As discussed in Chapter 1, Section 7.3.1, there are currently three herbicide tolerance traits in Australian canola varieties: non-GM TT (first available in 1993) and imidazolinone tolerant Clearfield® (first available in 1999) canola (GRDC, 2017), and GM glyphosate tolerant (GT, Roundup Ready® or TruFlex™ Roundup Ready®) canola, so hybrids with these are possible. If the GM juncea canola proposed for release were to cross with the TT, Clearfield® and GT canola, this could result in hybrids with tolerance to four herbicides, in a canola/juncea hybrid background. This has been a possibility since the approval of glufosinate tolerant InVigor® canola and Roundup Ready® canola in 2003, so approval of the GM juncea canola for commercial release would not add any new combinations of herbicide tolerance in hybrid volunteers. InVigor® (DIR 021/2002), InVigor® × Roundup Ready® canola (DIR 108) and InVigor® x TruFlex™ Roundup Ready® canola (DIR 138) have only been grown in breeding trials, so if the GM juncea canola proposed for release were widely grown in areas where those lines are growing, the likelihood of multiple-herbicide tolerant canola/juncea hybrids as volunteers could increase. In North America, where canola varieties that are tolerant to different herbicides are in close proximity, the production of multiple-herbicide tolerant volunteers has been noted (Hall et al., 2000; Beckie et al., 2003; Knispel et al., 2008; Schafer et al., 2011).

192. Crossing may also occur between the GM juncea canola and the male sterile MS11 canola approved for commercial cultivation under DIR 175 to produce fertile MS11 × RF3 hybrid. However, even if the hybridisation does happen, this hybrid would contain the same genes and traits as the MS11 × RF3 canola already approved for commercial release under DIR 178, in a canola/juncea hybrid background.

193. Cross-pollination between *B. juncea* and *B. rapa* could also occur in the field if plants of the two species are in proximity, but hybridisation is much less likely than with *B. napus* (Chapter 1, Section 7.3.1). Also, the chance of cross-pollination between *B. juncea* and other Brassica vegetables or forages is very low.

194. Based on the information above, hybridisation between the GM juncea canola and other Brassica crop species is expected to occur if the GM juncea canola is released. The likelihood of crossing between the GM juncea canola and canola crops is expected to be higher than the likelihood of crossing between the GM juncea canola and other Indian mustard plants, because canola is more widely grown than other Brassica crops (ABARES, 2015). Therefore, although the sexual compatibility

of *B. juncea* with *B. napus* is lower than between *B. juncea* plants, hybridisation between the GM juncea canola and other canola is still likely to occur at low levels. Hybridisation between the GM juncea canola and other Brassica species, with lower sexual compatibility, is expected to be at very low levels.

195. Volunteer plants that are hybrids between the GM juncea canola and other Brassica crop species could not be controlled by the application of glufosinate herbicides. However, the hybrid volunteers could be controlled by integrated weed management practices, which would include using a variety of other herbicides approved by the APVMA for use on Brassica volunteers, as well as non-chemical management methods currently used to control non-GM Brassica plants. As discussed in previous risk scenarios, the presence of the introduced genes is not expected to alter intrinsic characteristics contributing to spread and persistence, or to alter the tolerance of GM plants to biotic or abiotic stresses. Therefore, GM hybrid volunteers would not be expected to be more invasive or persistent than hybrids between non-GM juncea canola and other Brassica crop species.

Interactions with Brassicaceae weeds

196. Brassicaceous agricultural weeds are expected to be present in fields or field margins where the GM juncea canola would be grown, or in intensive use areas into which the GM juncea canola could be dispersed. Cross-pollination could occur if weeds are not destroyed prior to flowering, if there is synchronous flowering of weeds and the GM juncea canola, and if the weed species is sexually compatible with *B. juncea.*

197. Naturally occurring hybrids between *B. juncea* and weed species (wild radish, *Raphanus raphanistrum*; Buchan weed, *Hirschfeldia incana*; and charlock, *Sinapis arvensis*) have not been observed (Chapter 1, Section 7.3.1). Thus, introgression of the herbicide genes from the GM juncea canola into wild radish, Buchan weed and charlock populations is highly unlikely.

198. *B. juncea* has been reported to cross with other Brassicaceae weeds with human intervention, but not in open-pollination field conditions. Therefore, hybridisation between the GM juncea canola and other Brassicaceae weeds would be unlikely (OGTR, 2017).

199. In the unlikely event that herbicide tolerance gene was introgressed into populations of wild radish, Buchan weed or charlock, which retained the vigour of the recurrent weedy parent, these plants could establish as weeds. These GM weeds could not be controlled by the application of glufosinate herbicides. However, other weed management practices would be as effective on the GM weeds as they are on the parent non-GM weeds.

Potential harm

Interactions with Brassica crop species

200. Both volunteer juncea canola and other Brassica crop species are weeds of agricultural production systems (Groves et al., 2003). Any hybrids between the GM juncea canola and other Brassica species could also become volunteers. If left uncontrolled, GM hybrid volunteers could reduce the establishment or yield of desired crops.

201. Hybrid volunteers with multiple herbicide tolerance traits may not be effectively controlled by existing weed management measures, particularly where there is no awareness of herbicide tolerance traits acquired by pollen flow. However, additional herbicide tolerance traits are not expected to provide a survival advantage to the GM juncea canola, except in the presence of glufosinate herbicides. If they occurred, hybrid volunteers expressing all four currently available herbicide tolerance traits could be controlled by herbicides belonging to five other mode of action groups, or by non-chemical management practices, as discussed in Risk scenario 2. Multiple-herbicide tolerant individuals are as susceptible to alternative herbicides as single-herbicide tolerant canola plants (Beckie et al., 2004). Hybrid GM volunteers could be controlled by integrated weed management practices, which would include using other herbicides approved by the APVMA for use on Brassica

volunteers, as well as non-chemical management methods currently used to control non-GM Brassica plants.

Interactions with Brassicaceae weeds

202. Wild radish is a widespread serious agricultural weed, Buchan weed can be problematic in winter cereal crops, and charlock is primarily an agricultural or ruderal weed (Chapter 1, Section 7.3.1). If the GM herbicide tolerance trait was introgressed into a population of one of these weeds, it would increase the difficulty of weed management, particularly where there is no awareness of the herbicide tolerance trait. These GM weeds could impact the agricultural environment by reducing the establishment or yield of desired crops.

203. It should be noted that weeds can evolve herbicide resistance through natural mechanisms due to selective pressure. If these weeds did acquire a herbicide tolerance gene from the GM juncea canola, they would be no more difficult to control than those that had naturally evolved herbicide resistance, although there is currently no weed species with glufosinate resistance have been reported in Australia (Chapter 1, Section 7.3.4).

Conclusion

204. Risk scenario 5 is not identified as a substantive risk because hybrids between the GM juncea canola and Brassica crops or weed species would occur at low levels and multiple-herbicide tolerant hybrids can be controlled using integrated weed management. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

205. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis[12]. There are several types of uncertainty in risk analysis (Clark and Brinkley, 2001; Hayes, 2004; Bammer and Smithson, 2008). These include:

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

206. Uncertainty is addressed by approaches including balance of evidence, conservative assumptions, and applying measures that reduce the potential for 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.

207. RF3 juncea canola has been approved by the Regulator for limited and controlled release (field trial) under licences DIR 057/2004, DIR 069/2006 and DIR 104. The RARMPs for these field trials

[12] 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.

identified additional information that may be required for a large scale or commercial release of RF3 juncea canola. This includes the uncertainty associated with the potential for any unintended effects as a result of changes in biochemistry, physiology or ecology of the GM juncea canola plants, particularly noting further information related to enhanced tolerance to abiotic or biotic stress. Information provided by the applicant addressing these areas of uncertainty is presented in Chapter 1, Section 6.2, and discussed in relevant sections in Chapter 1 and in risk scenarios.

208. Uncertainty can arise from a lack of experience with the GMO. RF3 juncea canola proposed for release has only been approved in Australia under field trial licences. Also, non-GM *B. juncea* crops have only been grown in a small scale in Australia and there is a lack of information regarding *B. juncea* pollen movement in Australia (discussed in Risk Scenario 4), so uncertainty exists about the growing of RF3 juncea canola commercially on a large scale. However, the level of uncertainty is considered to be low, given that the GM juncea canola and its parental GM canola line, as well as earlier generation GM canola containing the *bar* and *barstar* genes have been grown as commercial crops in the USA and Canada for many years without any reported adverse effects on human health and safety or the environment. Additionally, there is no expectation that these genes would behave any differently in *B. juncea* than in *B. napus* and information from international field trials with the GM juncea canola indicate that the GM juncea and its non-GM juncea parent do not demonstrate any biologically significant differences across a range of agronomic and compositional parameters.

209. Overall, the level of uncertainty in this risk assessment is considered low and does not impact on the overall estimate of risk.

210. Post release review (PRR) will be used to address uncertainty regarding future changes to knowledge about the GMO or the receiving environment (Chapter 3, Section 4). PRR is typically required for commercial releases of GMOs, which generally do not have limited duration.

Section 4 Risk evaluation

211. 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.

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

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

213. Five risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. The level of risk for each scenario was considered negligible in relation to both the seriousness and likelihood of harm, and by considering both the short and long term. The principal reasons for these conclusions are summarised in Table 8.

214. The *Risk Analysis Framework* (OGTR, 2013), which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no controls are required to treat these negligible risks. The

Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment[13].

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

Chapter 3 Risk management plan

Section 1 Background

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

216. 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.

217. 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.

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

Section 2 Risk treatment measures for substantive risks

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

Section 3 General risk management

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

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

3.1 Applicant suitability

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

- any relevant convictions of the applicant

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

222. If a licence were issued, the conditions would include a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability.

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

3.2 Testing methodology

224. If a licence were issued, BASF would be required to provide a method to the Regulator for the reliable detection of the GMO, and the presence of the introduced genetic materials in a recipient organism. This instrument would be required prior to conducting any dealings with the GMO.

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

225. If a licence were issued, any person, including the licence holder, could conduct any permitted dealing with the GMO.

3.4 Reporting requirements

226. If issued, the licence would oblige the licence holder to report without delay any of the following to the Regulator:

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

227. The licence holder would also be obliged to submit an Annual Report containing any information required by the licence.

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

3.5 Monitoring for compliance

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

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

Section 4 Post release review

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

232. The Regulator engages in ongoing oversight of licences to take account of future findings or changes in circumstances. If a licence was issued, this ongoing oversight would be achieved through post release review (PRR) activities. The three components of PRR are:

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

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

4.1 Adverse effects reporting system

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

4.2 Requirement to monitor specific indicators of harm

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

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

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

237. The characterisation of the risk scenarios discussed in Chapter 2 did not identify any risks greater than negligible. Therefore, they were not considered substantive risks that warranted further detailed assessment. Uncertainty is considered to be low. No specific indicators of harm have been identified in this RARMP for application DIR 190. However, specific indicators of harm may also be identified during later stages, e.g. following the consideration of comments received on the consultation version of the RARMP, or if a licence were issued, through either of the other components of PRR.

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

4.3 Review of the RARMP

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

Section 5 Conclusions of the consultation RARMP

240. The risk assessment concludes that the proposed commercial release of RF3 juncea canola poses negligible risks to the health and safety of people or the environment as a result of gene technology.

241. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, if a licence were to be issued, general conditions are proposed to ensure that there is ongoing oversight of the release.

Chapter 4 Draft licence conditions

Section 1 Interpretations and Definitions

1. In this licence:

   (a) unless defined otherwise in this licence, words and phrases used in this licence have the same meaning as they do in the Act and the Gene Technology Regulations 2001;

   (b) words importing a gender include every other gender;

   (c) words in the singular number include the plural and words in the plural number include the singular;

   (d) expressions used to denote persons generally (such as “person”, “party”, “someone”, “anyone”, “no-one”, “one”, “another” and “whoever”), include a body politic or corporate as well as an individual;

   (e) references to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;

   (f) where a word or phrase is given a particular meaning, other grammatical forms of that word or phrase have corresponding meanings;

   (g) specific conditions prevail over general conditions to the extent of any inconsistency.

2. In this licence:

‘**Act**’ means the *Gene Technology Act 2000* (Cth) or the corresponding State legislation under which this licence is issued.

‘**GM**’ means genetically modified.

‘**GMO**’ means the genetically modified organism that is the subject of the dealings authorised by this licence.

‘**OGTR**’ means the Office of the Gene Technology Regulator.

‘**Regulator**’ means the Gene Technology Regulator.

Section 2 Licence conditions and obligations

3. This licence does not authorise dealings with the GMO that are otherwise prohibited as a result of the operation of State legislation recognising an area as designated for the purpose of preserving the identity of GM crops, non-GM crops, or both GM crops and non-GM crops, for marketing purposes.

4. This licence remains in force until it is suspended, cancelled or surrendered. No dealings with the GMO are authorised during any period of suspension.

5. The licence holder is BASF Australia Ltd.

6. Any person, including the licence holder, may conduct any permitted dealing(s) with the GMO.

7. All dealings with the GMO are permitted.

8. Dealings with the GMO may be conducted in all areas of Australia.

9. This licence authorises dealings with the GMO described in **Attachment A**.

2.1 General obligations of the licence holder

10. The licence holder must notify the Regulator as soon as practicable if any of its contact details change.

Note: please address correspondence to OGTR.M&C@health.gov.au.

Prior to issuing a licence, the Regulator considers suitability of the applicant to hold a licence. The following two conditions address ongoing suitability of the licence holder.

11. The licence holder must, at all times, remain an accredited organisation in accordance with the Act and must comply with its instrument of accreditation.

12. The licence holder must:

(a) inform the Regulator as soon as practicable after any of these events occur:

i. any relevant conviction of the licence holder; or

ii. any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment; or

iii. any event or circumstances that would affect the capacity of the licence holder to meet the conditions of the licence; and

(b) provide any information related to the licence holder's ongoing suitability to hold a licence, if requested by the Regulator, within the timeframe stipulated by the Regulator.

13. The licence holder must inform any person covered by this licence, to whom a particular condition of the licence applies, of the following:

(a) the particular condition (including any variations of it); and

(b) the cancellation or suspension of the licence; and

(c) the surrender of the licence.

2.2 Provision of new information to the Regulator

Licence conditions are based on the risk assessment and risk management plan developed in relation to the application using information available at the time of assessment. The following condition requires that any new information that may affect the risk assessment is communicated to the Regulator.

14. The licence holder must inform the Regulator if the licence holder becomes aware of:

(a) additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or

(b) any contraventions of the licence by a person covered by the licence; or

(c) any unintended effects of the dealings authorised by the licence.

Note: The Act requires, for the purposes of the above condition, that:

(a) the licence holder will be taken to have become aware of additional information of a kind mentioned in condition 14 if he or she was reckless as to whether such information existed; and

(b) the licence holder will be taken to have become aware of contraventions, or unintended effects, of a kind mentioned in condition 14, if he or she was reckless as to whether such contraventions had occurred, or such unintended effects existed.

Note: Contraventions of the licence may occur through the action or inaction of a person.

15. If the licence holder is required to inform the Regulator under condition 14, the Regulator must be informed without delay.

Note: An example of informing without delay is contact made within a day of becoming aware of new information via the OGTR free call phone number 1800 181 030, which provides emergency numbers for incidents that occur out of business hours.

16. If at any time the Regulator requests the licence holder to collect and provide information about any matter to do with the progress of the dealings authorised by this licence, including but not confined to:

(a) additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence, whether or not the licence holder has provided information to the Regulator under condition 14(a);

(b) any contraventions of the licence by a person covered by the licence, whether or not the licence holder has provided information to the Regulator under condition 14(b);

(c) any unintended effects of the dealings authorised by the licence, whether or not the licence holder has provided information to the Regulator under condition 14(c);

(d) research, including by way of survey, to verify predictions of the risk assessment, or for any purpose related to risks to the health and safety of people, or to the environment;

(e) scientific literature and reports in respect of the GMO authorised by this licence, for a nominated period;

(f) details of any refusals of applications for licences or permits (however described) to deal with the GMO made pursuant to the regulatory laws of a foreign country;

and the request is reasonable, having regard to consistency with the Act and relevance to its purpose, then the licence holder must collect the information and provide it to the Regulator at a time and in the manner requested by the Regulator.

Note: The Regulator may invite the licence holder to make a submission on the reasonability of a request by the Regulator to collect and provide information relevant to the progress of the dealings with the GMO.

2.3 Obligations of persons covered by the licence

17. If a person is authorised by this licence to deal with the GMO and a particular condition of this licence applies to the dealing by that person, the person must allow the Regulator, or a person authorised by the Regulator, to enter premises where the dealing is being undertaken, for the purposes of auditing or monitoring the dealing.

Section 3 Reporting and documentation

3.1 Annual Report

18. The licence holder must provide an annual report to the Regulator by the end of September each year covering the previous financial year. An annual report must include:

(a) information about any adverse impacts, unintended effects, or new information relating to risks, to human health and safety or the environment caused by the GMO or material from the GMO;

(b) information about the volumes of the GMO grown for commercial purposes, including seed increase operations, in each State and Territory for each growing season in the period;

(c) information about the volumes of the GMO grown for non-commercial (e.g. research) purposes in each State and Territory for each growing season in the period.

Note: nil plantings should also be reported under conditions 18(b) and 18(c).

3.2 Testing methodology

19. At least 14 days prior to conducting any dealings with the GMO, the licence holder must provide to the Regulator a written methodology to reliably detect the GMO, or the presence of the genetic modifications described in this licence in a recipient organism. The detection method(s) must be capable of identifying, to the satisfaction of the Regulator, each genetic modification event described in this licence.

Note: please address correspondence to OGTR.M&C@health.gov.au.

ATTACHMENT A

DIR No: 190

Full Title: Commercial release of Indian mustard genetically modified for herbicide tolerance (RF3 juncea canola)

Organisation Details

Postal address: BASF Australia Ltd
GPO Box 4705
Melbourne VIC 3001

Phone No: 03 8855 6600

GMO Description

GMO covered by this licence

Brassica juncea (L.) Czern. & Coss. genetically modified by the introduction of only the genes and genetic elements listed below.

Parent Organism

Common Name: Indian mustard (juncea canola)

Scientific Name: *Brassica juncea* (L.) Czern. & Coss.

Modified traits

Category: Herbicide tolerance
Male fertility restoration

Description: The GMO is the result of conventional breeding between GM RF3 canola, which is authorised for commercial release under licence DIR 021/2002, and a non-GM juncea canola line. The introduced genes and associated regulatory elements are listed in Table 1 of this attachment.

Purpose of the dealings with the GMO

The purpose of the dealings is commercial production of the GM juncea canola in all areas of Australia, and for products of the GMO to enter general commerce.

Table 1 Genetic elements responsible for conferring the modified traits

Gene (source)	Promoter (source)	Terminator (source)	Protein produced	Protein function
bar (*Streptomyces hygroscopicus*)	*PssuAt* (*Arabidopsis thaliana*)	*3' g7* (*Agrobacterium tumefaciens*)	PAT (phosphinothricin acetyl transferase)	Glufosinate tolerance
barstar (*Bacillus amyloliquefaciens*)	*PTa29* (*Nicotiana tabacum*)	*3'-nos* (*Agrobacterium tumefaciens*)	Barstar (RNase inhibitor)	Restoration of male fertility

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Appendix A: Summary of submissions

The Regulator received several submissions from prescribed experts, agencies and authorities[14] on matters relevant to preparation of the RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. These issues, and where they are addressed in the consultation RARMP, are summarised below.

Submission	Summary of issues raised	Comment
1	Agrees that the following matters should be considered in the RARMP: • potential for the GM Indian mustard to be harmful to people through toxicity or allergenicity • for the GM Indian mustard to be harmful to other organisms through toxicity • potential for the introduced traits to increase the weediness of the GM Indian mustard, leading to harm to the environment • potential for harm to result from gene flow to related species potential for commercial release to result in changes to agricultural practices that may have an adverse environmental impact.	The potential toxicity or allergenicity of the GM Indian mustard to people or toxicity to other organisms are addressed in Chapter 2, Section 2.4.1 (Risk scenario 1). The potential for increased weediness of the GM Indian mustard, leading to harm to the environment, is addressed in Chapter 2, Sections 2.4.2 and 2.4.3 (Risk scenarios 2 and 3). The potential for crossing between the GM Indian mustard and related species is addressed in Chapter 2, Section 2.4.5 (Risk scenario 5). Chapter 1, Section 7.1 discusses the agricultural practices for the GM Indian mustard that would not differ from standard industry practices except for the application of glufosinate. Environmental impacts of herbicides containing glufosinate are regulated by the APVMA.
2	Council has no commercial farming or Indian mustard growing areas in its jurisdiction and has no official policy on GM Indian mustard but would like its use to be undertaken in a way that is safe to both the public and the environment.	Noted.
3	Does not have specific advice on risks to the health and safety of people and the environment to be considered in the development of the consultation RARMP. Notes that there will be further opportunity for input into DIR 190 once the RARMP is made available for comment.	Noted.

[14] Prescribed experts, agencies and authorities include GTTAC, State and Territory Governments, relevant local governments, Australian government agencies and the Minister for the Environment.

Submission	Summary of issues raised	Comment
4	Notes that information from previous RARMPs for field trials in Australia of various GM *B. juncea* and GM *B. napus* may be relevant in the preparation of the RARMP.	Noted. As discussed in Chapter 2, Section 1, risk scenarios in previous RARMPs prepared for the same or similar GMOs are considered when postulating risk scenarios.
	Comments that while there is currently no conclusive evidence of environmental harm, there is a lack of environmental risk assessment (ERA) data on GM canola and GM *B. juncea* especially under Australian conditions. RARMPs and related advice on field trials of GM *B. juncea* published since 2004 have repeatedly requested data be collected on persistence, weediness and gene flow to weedy relatives under Australian conditions if a commercial release were to be considered. Like canola, *B. juncea* has weedy characteristics and while it is not classified as an environmental weed in Australia, it is classified as an environmental weed in the US and has several weedy relatives that are environmental weeds in Australia and overseas. Gene flow can occur between GM *B. juncea* and the weedy relative, *B.rapa*.	The biology document for *B. napus* and *B. juncea* (OGTR, 2017) includes a weed risk assessment for each species. This is discussed in the RARMP (Chapter 1, Section 4.2), together with information from extensive literature reviews. Additional consideration of this material is also included in the risk scenarios (Risk scenarios 2 and 3). The potential for gene flow from the GM juncea canola is considered in Chapter 1, Sections 7.3.1 and 7.3.2, and Chapter 2, Sections 2.4.4 and 2.4.5 (Risk Scenarios 4 and 5) of the RARMP, based on data and information from a range of sources.
	Recommends the RARMP includes the following: • information relevant to environmental risk assessment on dispersal, survival, weediness, fitness advantage and gene flow to weedy relatives. • clearly note the greater uncertainty with GM *B. juncea* compared to GM canola and the lack of Australian data. risk management measures or licence conditions to collect ERA data on dispersal, persistence, abiotic stress tolerance, and gene flow under Australian conditions, to address the uncertainty and to better inform future risk assessment and management.	Noted. See specific comments below.
	Dispersal The RARMP should discuss seed dispersal ability of *B. juncea* seed and include a discussion on the experience in Canada and the US. *B. juncea* may appear as less of a problem due to the considerably lower amounts of *B. juncea* grown in Canada compared to canola.	Seed dispersal outside of cultivation is considered and discussed in the Chapter 2, Section 2.4.3 (Risk scenario 3). Weed risk potential of *B. juncea* is discussed in Chapter 1, Section 4.2.
	Survival The RARMP for DIR 190 should include data to support that *B. juncea*: • has limited ability to spread, persist or establish outside cultivation	Relevant data have been included in Chapter 1, Section 6.2.3, and in Risk scenario 3.

Submission	Summary of issues raised	Comment
	• is still a poor competitor compared with native species and does not establish well in non-managed areas it is not considered competitive and volunteers less frequent than canola.	
	Weediness The weediness status of *B. juncea* should be assessed in the RARMP with the inclusion of the following information: • related Brassica species that are environmental weeds • overseas weediness Australian plant databases for occurrence records.	The weediness status of *B. juncea* globally and in Australia is discussed in Chapter 1, Section 4.2.
	Fitness advantage To adequately assess potential environmental risks, the RARMP should include all of the available relevant information that both supports or refutes any potential for the trait of herbicide tolerance to alter fitness in the GM plants.	The potential for increased fitness due to the introduced glufosinate tolerance gene in the GM juncea canola proposed for release is discussed in Risk scenario 3. The data provided for the GMO and its non-GM parent with respect to abiotic and biotic stress and plant growth characteristics are discussed in Chapter 1, Section 6.2.3.
	The RARMP should include confined field trial data, and its transferability and adequacy, comparing abiotic and biotic stress tolerance in GM and non-GM *B. juncea* from field trials conducted in 2017 in the US and Canada.	Such data have been included in Chapter 1, Section 6.2.3. Transferability of field trial data from Canada and the USA for this application is discussed in Chapter 1, Section 6.2.3.
	Uncertainty Suggests that the RARMP recommends the applicant gather relevant field trial data on competitiveness and abiotic stress tolerance of GM *B. juncea* under Australian conditions, to reduce uncertainty about the environmental risks and better inform both the risk assessment and appropriate risk management measures.	Uncertainty concerning lack of experience with large scale cultivation of this GMO is discussed in Chapter 2, Section 3. However, because of the overseas data, the level of uncertainty is considered low and therefore collection of Australian data is not considered necessary. The RARMP concludes that the proposed commercial release of RF3 juncea canola poses negligible risks to the health and safety of people or the environment, and no specific risk treatment measures will be required but general conditions are proposed to ensure that there is ongoing oversight of the release.
5	No comment.	Noted.