Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 178

Commercial release of canola genetically modified for herbicide tolerance and a hybrid breeding system (MS11 × RF3 and MS11 × RF3 × MON 88302)

Applicant: BASF Australia Ltd

September 2021

PAGE INTENTIONALLY LEFT BLANK

Summary of the Risk Assessment and Risk Management Plan

for

Licence Application No. DIR 178

Decision

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

The application

Application number	DIR 178
Applicant	BASF Australia Ltd (BASF)
Project title	Commercial release of canola genetically modified for herbicide tolerance and a hybrid breeding system (MS11 × RF3 and MS11 × RF3 × MON 88302)[1]
Parent organism	*Brassica napus* L. (canola)
Introduced genes and modified traits	**Two genes for herbicide tolerance:** • *bar* gene from *Streptomyces hygroscopicus* for glufosinate tolerance • *cp4 epsps* gene from *Agrobacterium* sp. strain CP4 for glyphosate tolerance **Two genes for a hybrid breeding system:** • *barnase* gene from *Bacillus amyloliquefaciens* for male sterility • *barstar* gene from *Bacillus amyloliquefaciens* for fertility restoration
Proposed locations	Australia-wide
Primary purpose	Commercial release for canola 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

[1] The title of the application submitted by BASF is "Commercial release of MS11 × RF3 *B. napus* and MS11 × RF3 x MON 88302 *B. napus* in the Australia cropping system, genetically modified for herbicide tolerance and a hybrid breeding system".

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

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

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

Risk management

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

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

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

Table of contents

Abbreviations

ABARES	Australian Bureau of Agricultural and Resource Economics and Sciences
the Act	The *Gene Technology Act 2000*
ANZFA	Australia New Zealand Food Authority
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
bp	Base pair
CANBR	Centre for Australian National Biodiversity Research
CCI	Confidential Commercial Information under section 185 of the *Gene Technology Act 2000*
CMP	Crop management plan
cp4 epsps	*epsps* gene from Agrobacterium sp. strain CP4
CP4 EPSPS	EPSPS protein from Agrobacterium sp. strain CP4
CTP	Chloroplast transit peptide
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
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Global Food and Agriculture Statistics of FAO
FMV	Figwort mosaic virus
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
HGT	Horizontal gene transfer
IMI	Imidazolinone tolerant
ISAAA	International Service for the Acquisition of Agri-Biotech Applications
kDa	Kilodalton(s)
km	Kilometre(s)
LOQ	Limit of quantification

LLOQ	Lower limit of quantification
m	Metre(s)
µg	Microgram(s)
mg	Milligram(s)
mL	Millilitre(s)
µm	Micrometre(s)
µmol	Micromole(s)
mol	Mole(s)
mRNA	Messenger ribonucleic acid
MS	Male sterile
NAG	*N*-acetyl-L-glufosinate
ND	Not determined
ng	Nanogram(s)
NSW	New South Wales
NZ	New Zealand
MPP	3-methyl phosphinico-propionic acid
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
SA	South Australia
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
WA	Western Australia
WHO	World Health Organization

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.

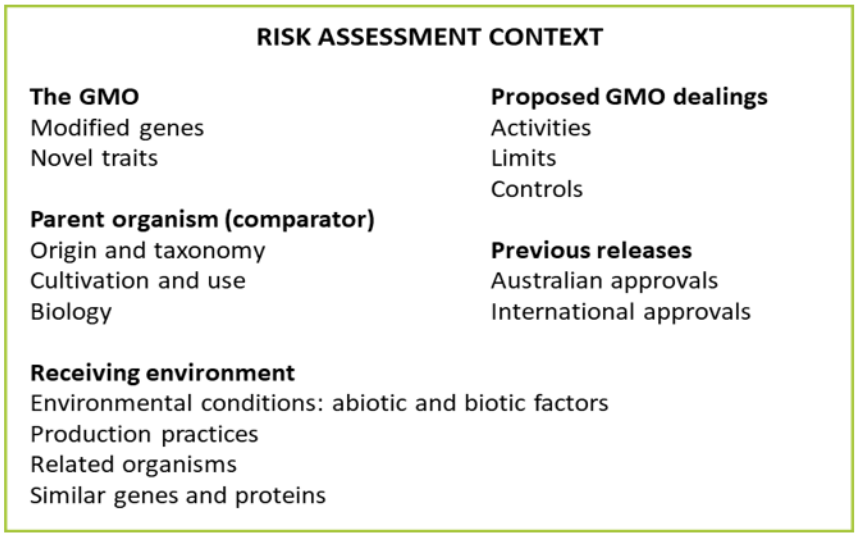

Figure 1 Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.

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. Advice from the prescribed experts, agencies and authorities in the second round of consultation, and how it was taken into account, is summarised in Appendix B. Four public submissions were received and their consideration is summarised in Appendix C.

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.* FSANZ has approved food derived from MS11 canola as safe for human consumption (FSANZ, 2017).

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 genetically modified (GM) canola lines (MS11 × RF3 and MS11 × RF3 × MON 88302). MS11 × RF3 was developed by conventional breeding between the two GM canola lines MS11 and RF3 and contains two introduced genes for a hybrid breeding system and one introduced gene that confers tolerance to herbicides containing glufosinate. This line is also known by the unique OECD identifier (BCS-BNØ12-7 x ACS-BNØØ3-6).
MS11 × RF3 × MON 88302 was the result of conventional breeding among MS11, RF3 and the MON 88302 canola and contains the same genes for a hybrid breeding system and glufosinate tolerance plus another introduced gene for tolerance to herbicides containing glyphosate. This line is also known by the unique OECD identifier (BCS-BNØ12-7 x ACS-BNØØ3-6 x MON-88302-9).

[2] BASF is seeking approval for unrestricted commercial release of the GM canola lines in all canola growing areas of Australia. Canola may be grown over a significant proportion of Australian agricultural land, and viable seed may be transported out of the canola growing areas. Therefore, the Regulator decided to consult with all of the local councils in Australia, except for those that have requested not to be consulted on such matters.

14. BASF also proposes to release the two intermediate parental lines MS11 x MON 88302 and RF3 x MON 88302, created through conventional breeding, as these lines would be used in breeding and seed multiplication process for MS11 × RF3 × MON 88302.

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

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

(a) conduct experiments with the GMOs

(b) make, develop, produce or manufacture the GMOs

(c) breed the GMOs

(d) propagate the GMOs

(e) use the GMOs in the course of manufacture of a thing that is not the GMOs

(f) grow the GMOs

(g) import the GMOs

(h) transport the GMOs

(i) dispose of the GMOs

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

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

3.1 Australian approvals

3.1.1 GMOs proposed for release

17. MS11 × RF3 has been approved by the Regulator for limited and controlled release under licences DIR 069/2006 and DIR 104. MS11 × RF3 × MON 88302 has not previously been approved for intentional release into the environment in Australia, but a substantially similar stack (MS8 × RF3 × MON 88302 has been approved under DIR 138 (see Table 1).

3.1.2 Parental GM canola lines

18. All three parental events have been previously assessed (individually and/or in combination with other events) and authorised for commercial release by the Regulator.

GM parent MS11 canola

19. The Regulator has previously authorised canola with the MS11 event for limited and controlled release under licences DIR 069/2006 and DIR 104, as well as for commercial cultivation under the recently issued licence DIR 175.

GM parent RF3 canola

20. The Regulator has previously authorised canola with the RF3 event for limited and controlled release under licences DIR 069/2006 and DIR 104, as well as for commercial cultivation under the licence DIR 021/2002.

GM parent MON 88302 (TruFlex™ Roundup Ready® canola)

21. Field trials of MON 88302 canola have been conducted in Australia since 2011 under licence DIR 105. Commercial release of MON 88302 canola was approved by the Regulator in November 2014 under the licence DIR 127. As yet it has not been grown on a commercial scale in Australia.

22. Previous assessments of MS11, RF3 and MON 88302 canola concluded that these events pose negligible risks to human health and safety, and the environment. There have been no reported adverse effects on human health or the environment resulting from any of these releases.

3.1.3 Other relevant GM canola

23. A number of licences have been issued for canola with a hybrid breeding system and herbicide tolerance (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 hybrid breeding system and herbicide tolerance traits.

Table 1 Previous approvals of canola with an introduced hybrid breeding system and herbicide tolerance for intentional release in Australia

DIR licence number	Licence type	Title	Relevant modified traits
010/2001*	L&C[a]	Small and large scale trialing of InVigor® canola (*Brassica napus*) for the Australian cropping system and seed production	HBS[c], glufosinate tolerance
032/2002*	L&C	Field trial - Seed increase and field evaluation of herbicide tolerant genetically modified canola incorporating a hybrid breeding system	HBS, glyphosate tolerance
108[#]	C[b]	Commercial release of canola genetically modified for herbicide tolerance and a hybrid breeding system (InVigor® x Roundup Ready® canola)	HBS, glufosinate and glyphosate tolerance
138[#]	C	Commercial release of canola genetically modified for dual herbicide tolerance and a hybrid breeding system (InVigor® x TruFlex™ Roundup Ready®)	HBS, glufosinate and glyphosate tolerance
175[#]	C	Commercial release of canola (*Brassica napus*) genetically modified for herbicide tolerance and a hybrid breeding system (MS11)	HBS, glufosinate tolerance

[a] L&C, limited and controlled release; [b] C, commercial release; [c] HBS, hybrid breeding system; * Surrendered; [#] Current

3.2 Approvals by other Australian agencies

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

25. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has approved food derived from MS11 (FSANZ, 2017), RF3 (ANZFA, 2001a) and MON 88302 canola (FSANZ, 2013b) as safe for human consumption. According to the FSANZ regulatory approach to food from stacked GM plants, no separate approval is required for foods derived from a stacked GM line that is the result of traditional breeding between two or more GM parent lines for which food has already

been approved. Therefore, the above approvals also cover the stacked events through conventional breeding in MS11 × RF3 and MS11 × RF3 × MON 88302 canola.

26. 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). Registration for the use of herbicides containing glyphosate would be needed if the GM canola line MS11 × RF3 × MON 88302 were approved for commercial cultivation in Australia.

3.3 International approvals

3.3.1 GMOs proposed for release

27. BASF has submitted applications to authorities in a number of countries for food or feed use, or cultivation of MS11 × RF3 and MS11 × RF3 × MON 88302. To date, Canada and Korea have approved these two GM canola lines as detailed in Table 2.

Table 2 International approvals of MS11 × RF3 and MS11 × RF3 × MON 88302

Country	MS11 x RF3			MS11 x RF3 x MON 88302		
	Food	Feed	Cultivation	Food	Feed	Cultivation
Canada		√	√		√	√
Korea	√	√		√		

3.3.2 Parental GM canola lines

28. A number of countries have approved the parental lines MS11, RF3 and MON 88302 for commercial cultivation, as well as food and feed use (Table 3).

Table 3 International approvals of MS11, RF3 and MON 88302

Country	MS11	RF3	MON 88302
Canada	√ (food, feed, cultivation)	√ (food, feed, cultivation)	√ (food, feed, cultivation)
China		√ (food, feed)	√ (food, feed)
EU		√ (food, feed)	√ (food, feed)
Japan		√ (food, feed, cultivation)	√ (food, feed, cultivation)
Mexico		√ (food)	√ (food)
New Zealand	√ (food)	√ (food)	√ (food, feed)
Philippines	√ (food, feed)	√ (food, feed)	√ (food, feed)
Singapore			√ (food)
South Korea	√ (food)	√ (food, feed)	√ (feed)
Taiwan	√ (food)	√ (food)	
USA	√ (food, feed, cultivation)	√ (food, feed, cultivation)	√ (food, feed, cultivation)

Source: ISAAA GM approval database; accessed February 2021

29. 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 release of the GM canola lines.

Section 4 The parent organism

30. The parent organism is canola (*Brassica napus* L.), which is commonly also known as rapeseed or oilseed rape. The species belongs to the Brassicaceae family, along with cruciferous vegetable crops, weedy species and ornamental plants (OGTR, 2017).

31. *Brassica napus* has a tetraploid genome (AACC, haploid chromosome number [*n*] = 19) formed via allopolyploidy between two diploid ancestors, *B. oleracea* (CC, *n* = 9) and *B. rapa* (AA, *n* = 10) (Chalhoub et al., 2014; OGTR, 2017).

32. *Brassica napus* is predominantly self-pollinating, but outcrossing can be mediated by insects, wind or physical contact. The rate of cross-fertilisation between plants averages around 30% (Hüsken and Dietz-Pfeilstetter, 2007). Cross-fertilisation is most likely to occur over short distances (less than 10 m), declining with increased distance; however low-level long-distance pollen flow has been reported at 2.5 km (OGTR, 2017).

33. *Brassica napus* pollen grains are large (32–35 µm) and sticky (Hüsken and Dietz-Pfeilstetter, 2007). The flowers contain nectar rich in sugar, which is attractive to bees (OGTR, 2017). Different taxa of bees and flies are effective pollinators of *B. napus*, with some beetle species capable of pollinating *B. napus* to a lesser extent (OGTR, 2017; Phillips et al., 2018). The relative contributions of wind and insects to the mediation of cross-pollination depends on seasonal conditions and insect abundance.

34. Isolation distances for the production of certified non-hybrid canola seed and other Brassicaceae are relatively large, compared with other crop species. Basic and certified seed production areas for canola must be 200 m and 100 m, respectively, from sexually compatible species (Seed Services Australia, 2013).

35. One *B. napus* plant can produce hundreds of small seeds, with each seed weighing approximately 3–6 mg (GRDC, 2015b; OGTR, 2017). Larger seeds, such as those produced by hybrid varieties, tend to be more vigorous and lead to better crop establishment.

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

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

4.1 Canola as a crop

38. Canola is exotic to Australia and is grown as an agricultural crop, mainly in Western Australia (WA), New South Wales (NSW), Victoria and South Australia (SA). It is Australia's third largest broadacre crop (ABARES, 2020).

39. Rapeseed was first cultivated commercially in Australia in the 1960s (Colton and Potter, 1999). Low erucic acid varieties of rapeseed, known as canola, were developed in the 1970s. The area sown to canola in Australia increased considerably in the 1990s with the introduction of improved varieties, agronomic developments and good prices (Colton and Potter, 1999), peaking in 2013 with over 3 million ha harvested (FAOSTAT website, accessed July 2020).

40. Canola seed is crushed to produce oil, which is used predominantly as cooking oil or in food products (GRDC, 2017a). 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 (OECD, 2011). Information on the use of the parent organism in agriculture is summarised in Section 7 (the receiving environment).

4.2 Weed risk potential for canola outside cultivation

41. *Brassica napus* is not recorded in the *Weeds of National Significance* list (Weeds Australia website, accessed April 2021), the *National Environmental Alert List* (Weeds Australia website, accessed April 2021) or the Noxious Weed List for Australian States and Territories (Invasive Plants and Animals Committee, 2015).

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

4.2.1 Potential to cause harm

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

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

44. *Brassica napus* 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).

45. 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). The Australian canola crop grown in 2018 contained on average less than 0.1% erucic acid in the oil and approximately 15 µmol/g of glucosinolates in the meal (Graham et al., 2019).

4.2.2 Invasiveness

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

- the ability to reproduce by seed, but not by vegetative means
- short time to seeding
- high annual seed production 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

47. Volunteer canola 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). Canola volunteers produce allelopathic compounds that reduce germination of other crops, in addition to directly competing with other plants (Asaduzzaman et al., 2020).

48. Due to its primary colonising nature, canola can take advantage of disturbed habitats such as roadside verges, field margins, wastelands and along railway lines. However, canola is a poor competitor with weed species and will be displaced unless the habitats are disturbed on a regular basis (Salisbury, 2002; OECD, 2012). The ability of spilled canola seed to establish is determined by many factors, including fine-scale environmental differences, and both intra- and interspecific genotypic variation (Meffin et al., 2018).

49. Feral canola plants are often observed growing on roadsides or railway easements in Australia; in the case of roadside canola, plants are typically within 5 m from the edge of the road (Agrisearch, 2001; Norton, 2003). Roadside canola populations are usually transient, and are thought to be reliant on re-supply of seed through spillages (Crawley and Brown, 2004).

50. Canola is not considered a significant weed in natural undisturbed habitats in Australia (Dignam, 2001; Groves et al., 2003). Canola seed burial in undisturbed habitats is likely very low, which may limit the potential for feral canola populations to persist in the seedbank via secondary dormancy (Busi and Powles, 2016).

4.2.4 Management of volunteer canola

51. Canola volunteers generally emerge in the year following a canola crop, but may emerge for up to three years in Australia (Australian Oilseeds Federation, 2019), with the seedbank declining rapidly (Baker and Preston, 2008). However, persistence of canola volunteers has been observed for up to 7 years in Canada (Beckie and Warwick, 2010) and 15 years in Germany (Belter, 2016).

52. The method for control of canola volunteers depends on the situation (Australian Oilseeds Federation, 2019). 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, including Group N (glufosinate), are currently available for the control of canola volunteers in Australia (APVMA PubCRIS database, accessed October 2020). Volunteer canola is most easily controlled at the seedling stage.

Section 5 The parental GM canola lines – nature and effect of genetic modification

53. The GM canola lines proposed for release are the results of conventional breeding among the parental GM canola lines MS11, RF3 and MON 88302.

5.1 The genetic modifications of the parental GM lines

54. The introduced genetic material, source organisms and traits are summarised in Tables 4 and 5.

Table 4 The traits and genes introduced into the parental GM canola lines

Parental GM canola line	Hybrid breeding system	Glufosinate tolerance	Glyphosate tolerance
MS11	*barnase* *barstar* (not expressed in anthers)	*bar*	-
RF3	*barstar* (2 copies)	*bar*	-
MON 88302	-	-	*cp4 epsps*

Table 5 Genetic elements and their origin

Gene (source)	Promoter (source)	Terminator (source)	Additional elements (source)	Protein produced	Protein function
bar (*S. hygroscopicus*)	*PSsuAra* (*A. thaliana*)	*3' g7* (*A. tumefaciens*)	-	PAT (phosphinothricin acetyl transferase)	Glufosinate tolerance
barnase (*B. amyloliquefaciens*)	*PTa29* (*N. tabacum*)	*3'-nos* (*A. tumefaciens*)	-	Barnase (RNase)	Male sterility
barstar (*B. amyloliquefaciens*)	*PTa29* (*N. tabacum*)	*3'-nos* (*A. tumefaciens*)	-	Barstar (RNase inhibitor)	Restoration of fertility
	Pnos (*A. tumefaciens*)	*3' g7* (*A. tumefaciens*)	-	Barstar (RNase inhibitor)	Enhancing trans-formation efficiency
cp4 epsps (*Agrobacterium* sp. strain CP4)	*P-FMV/Tsf-1* (FMV and *A. thaliana*)	*E9 3'* (*P. sativum*)	L-Tsf1 (leader sequence) & I-Tsf1 (intron) Ctp2 (chloroplast transit peptide) (*A. thaliana*)	CP4 EPSPS (5-enolpyruvylshikimate -3-phosphate synthase)	glyphosate tolerance

5.1.1 Method of genetic modification of the parental GM canola lines

55. All three parental GM canola lines were developed using *Agrobacterium tumefaciens*-mediated transformation. This method has been widely used in Australia and overseas for introducing genes into plants. More information can be found in the document *Methods of Plant Genetic Modification* on the Risk Assessment References page on the OGTR website. Details regarding the development of MS11, RF3 and MON 88302 using this method are provided in the RARMPs for DIR 175, DIR 021/2006 and DIR 127, respectively.

5.1.2 Hybrid breeding system

56. Traditional plant breeding selects for plants with agronomically valuable characteristics. However, repetitive self-pollination of desirable lines can produce progeny that display lowered fitness or vigour when compared to their out-crossing counterparts, a phenomenon termed inbreeding depression. By

contrast, when crosses are made between genetically distinct parents, the progeny often outperform the parental lines (e.g. exhibiting greater growth and yield) and are said to display hybrid vigour (or heterosis), a well-known biological phenomenon. Hybrid vigour is commercially advantageous, but the generation of hybrid seed poses challenges in crop species that are predominantly self-pollinated (Perez-Prat and van Lookeren Campagne, 2002). Self-pollination occurs in plants with hermaphrodite flowers, which have both male and female floral organs. In order to achieve 100% cross-pollination between two homozygous (inbred) lines, a pollination control breeding system is required. Typically, this involves the development of a male-sterile inbred line that receives pollen from a second inbred line during hybrid seed production.

57. For the current application, the hybrid breeding system is conferred by expression of the *barnase* and *barstar* genes derived from the common soil bacterium *Bacillus amyloliquefaciens*. The *barnase* gene encodes a 12 kilodaltons (kDa) ribonuclease (RNase), Barnase (110 amino acids), and the *barstar* gene encodes a 10 kDa RNase inhibitor protein, Barstar (89 amino acids), which specifically binds to Barnase and suppresses its activity (Hartley, 1988, 1989). Further details of the hybrid breeding system can be found in the RARMP for DIR 175 (OGTR, 2021).

58. RNases are commonly found in nature. Their function is to catalyse the cleavage of RNA in various processes, including the regulation of gene expression and microbial defence mechanisms (Yang, 2011). In *B. amyloliquefaciens*, Barnase is secreted extracellularly, where it is expected to have bactericidal activity, possibly towards bacteria of the same species (Ulyanova et al., 2011). Barstar accumulates intracellularly to protect the host cell from the destructive properties of its own ribonuclease enzyme (Hartley, 1988).

MS11

59. The MS11 line contains both the *barnase* and *barstar* genes. The *barnase* 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 results in localised degradation of ribonucleic acid within the tapetal cells prior to microspore development and prevents the production of pollen (Mariani et al., 1990; De Block and Debrouwer, 1993). The flowers of MS11 plants are male-sterile (MS) and can only be fertilised by the pollen of another plant, thereby ensuring the production of outcrossed progeny. The terminator (mRNA polyadenylation signals), is provided by the 3' non-translated region of the nopaline synthase gene (3'-nos) from *Agrobacterium tumefaciens* (Depicker et al., 1982).

60. The *barstar* gene is expressed constitutively at low levels under control of the Pnos promoter (Depicker et al., 1982; Michiels et al., 1996). The applicant states that the *barstar* gene is included as a prophylactic gene to enhance transformation frequency, and that expression of *barstar* is not sufficient to restore male fertility in MS11 canola. Although the Pta29 promoter is considered tapetum-specific, leaky expression of genes under the control of the Pta29 promoter has been implicated in cell death during regeneration of plants following transformation (Baldacci-Cresp et al., 2016). The expression of *barstar* driven by the weak Pnos promoter could limit the negative effects of leaky expression of the *barnase* gene in undifferentiated plant tissues.

61. Inheritance of the male sterility during maintenance of MS11 and commercial hybrid seed production is described in the RARMP for DIR 175 (OGTR, 2021).

RF3

62. To reverse the effects of *barnase* expression, the GM canola line RF3 has been generated that contains the *barstar* gene. The introduced *barstar* gene in RF3 is under the control of the same regulatory sequences as the *barnase* gene in MS11. Expression of *barstar* has no effect on pollen development and GM canola plants have a normal appearance and viable pollen (Mariani et al., 1992). When MS11 containing *barnase* is crossed with RF3 containing *barstar*, progeny that inherit both genes display completely normal fertility due to the specific inhibition of Barnase activity by Barstar in the

tapetal cell layer of the anthers (Mariani et al., 1992). The RF3 canola modified with the *barstar* gene is therefore a restorer of fertility.

5.1.3 Herbicide tolerance

Glufosinate tolerance

63. Glufosinate is the active ingredient in a number of Group N herbicides (GRDC, 2017b). These herbicides function by inhibiting the plant enzyme glutamine synthase, which is a key enzyme involved in plant nitrogen metabolism. In the absence of glutamine synthase activity, ammonia accumulates in plant tissues causing inhibition of amino acid biosynthesis, inhibition of photosynthesis and rapid death of the plant (Evstigneeva et al., 2003). Glufosinate (also known as phosphinothricin) is an L-glutamic acid analogue, which is a component of the tripeptide bialaphos, an antibiotic secondary metabolite produced by the soil-borne bacterium *Streptomyces hygroscopicus* (Murakami et al., 1986).

64. Both the MS11 and RF3 lines 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. 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). Expression of the *bar* gene in MS11 and RF3 is controlled by the plant promoter PssuAt from the ribulose-1,5-bisphosphate carboxylase (RubisCO) small subunit gene from *Arabidopsis thaliana*, which directs gene expression in green plant tissues (Krebbers et al., 1988).

65. The *bar* gene introduced into MS11 and RF3 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).

66. The terminator for the *bar* gene in MS11 and RF3 is 3'g7, derived from the 3' non-translated region from gene 7 of *A. tumefaciens* found in octopine tumours of tobacco after bacterial infection (Dhaese et al., 1983; Velten and Schell, 1985).

Glyphosate tolerance

67. Glyphosate (N-phosphonomethyl glycine) is the active ingredient in a number of Group M herbicides (GRDC, 2017b). The herbicidal activity of glyphosate is derived from its ability to inhibit the function of 5 enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme involved in the shikimate biosynthetic pathway present in all plants, bacteria and fungi. Glyphosate competes with phosphoenolpyruvate for binding to the complex formed between EPSPS and shikimate 3 phosphate. Upon glyphosate binding, the EPSPS:shikimate 3-phosphate complex is highly stable and has a slow reversal rate, effectively terminating the shikimate pathway prematurely and preventing biosynthesis of essential aromatic compounds, including the amino acids phenylalanine, tyrosine and tryptophan, and eventually leading to cell death (Dill, 2005).

68. The CP4 EPSPS protein encoded by the *cp4 epsps* gene from *Agrobacterium* sp. is largely insensitive to the effects of glyphosate (Padgette et al., 1993). Consequently, in GM plant cells with the Agrobacterium *cp4 epsps* gene, biosynthesis of aromatic amino acids is not inhibited in the presence of glyphosate. Therefore, no new metabolic products are formed in these GM plants as the only difference from the native EPSPS enzyme is the reduced affinity for glyphosate (OECD, 1999a).

69. MON 88302 canola was modified by the insertion of the *cp4 epsps* gene, which encodes an EPSPS protein consisting of 455 amino acids (Padgette et al., 1996). EPSPS is a key enzyme in plants, bacteria, algae and fungi but is absent from mammals, birds, reptiles and fish which are not able to synthesize these aromatic amino acids (Bentley, 1990; Padgette et al., 1993). Further detailed description of the CP4 EPSPS protein and its function in MON88302 canola can be found in the RARMP for DIR 127.

5.1.4 Molecular characterisation of the GM parental lines MS11, RF3 and MON 88302

70. The exact location of the insert in the parental MS11, RF3 and MON 88302 canola are not known. However, molecular characterisation of these parental lines has been carried out using Southern blot and PCR analyses, as well as molecular cloning and sequencing of the site of insertion for each of these lines. Stable integration and inheritance of the inserted T-DNA was demonstrated in all the parental lines. DNA sequencing was used to verify the inserted genes and to determine the regions flanking all of the insertion sites.

71. In MS11 and MON 88302, a single insertion event occurred resulting in transfer of a single copy of the T-DNA (Monsanto Company, 2010; Anon., 2016). In RF3, previous data provided to the OGTR indicated that a single insertion event occurred that resulted in the integration of one complete T-DNA copy and a second, incomplete T-DNA copy arranged in an inverted repeat configuration (OGTR, 2003). However, updated information indicated that although the configuration of the single insertion event remains unchanged, the two T-DNAs are both incomplete with one T-DNA containing a complete *bar* gene cassette and a truncated *barstar* gene cassette and the other T-DNA containing only a complete *barstar* gene cassette without the *bar* gene (information provided by the applicant).

72. In multiple field trials, breeding programs and seed production, there have been no reports of aberrant segregation and instability for either MS11, RF3 or MON 88302 canola.

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

Barnase and Barstar proteins

73. The parental GM lines have been approved for food and feed use as well as environmental release in Australia and overseas with no credible reports of adverse effects (Section 3).

74. Barnase acts as a bacteriocin and evidence suggests that this enzyme may be a mechanism for *B. amyloliquefaciens* to acquire nutrients (Ulyanova et al., 2011). These cytotoxic effects are exploited, via GM strategies, to produce various traits in plants (including male sterility) and have also been investigated in cancer research.

75. 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.

76. The *barnase–barstar* hybrid breeding system has been extensively assessed in previous RARMPs for commercial release of GM canola (DIR 021/2002, DIR 108, DIR 138 and DIR 175). The Barnase and Barstar proteins have been assessed to lack toxicity to humans or animals, or allergenicity in humans on the following basis:

- the *barnase* and *barstar* genes were obtained from the common soil bacterium *B. amyloliquefaciens*, which is used as a source of enzymes for food industries and not known to be allergenic or pathogenic towards humans
- no sequence homology has been found between Barnase or Barstar and known toxins or allergens
- Barnase or Barstar do not have characteristics typical of known protein allergens
- Barnase and Barstar are both rapidly degraded in simulated gastric juices, with complete protein degradation within five minutes, showing that these proteins would not easily survive in the digestive tract
- feeding studies in rabbits, canaries and broiler chickens have shown that MS × RF canola lines (containing Barnase and Barstar) are nutritionally equivalent to non-GM canola.

77. 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 ubiquitously present in the environment (can be found in water, soil, air, decomposing plant material and on fresh produce).

78. FSANZ has approved food derived from GM canola lines expressing Barnase and Barstar proteins as safe for human consumption (ANZFA, 2001b; FSANZ, 2017).

PAT protein

79. 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 and DIR 138) 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 soil bacterium *S. hygroscopicus*, which is not considered a pathogen of humans or other animals
- no sequence homology has been found between PAT and any known toxic or allergenic proteins
- the PAT protein does not possess any of the characteristics associated with food allergens
- the PAT protein is inactivated by heat, e.g. through cooking, and by low pH, e.g. in the human stomach
- the PAT protein is rapidly degraded in simulated gastric or intestinal fluid
- purified PAT protein was not toxic to mice and rats when administered at high doses in acute toxicity studies.

80. 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, 2001b; FSANZ, 2017), cotton (FSANZ, 2005a, 2010a, b, 2013a), corn (FSANZ, 2005c) and rice (FSANZ, 2008).

CP4 EPSPS protein

81. The *cp4 epsps* gene and its encoded CP4 EPSPS protein have been extensively assessed in previous RARMPs for commercial release of GM crops including canola (DIR 108, DIR 127 and DIR 138) and cotton (DIR 118, DIR 124 and DIR 145). The CP4 EPSPS protein has been assessed to lack toxicity to humans or animals, or allergenicity in humans on the following basis:

- the *cp4 epsps* gene is derived from the common soil bacteria, *Agrobacterium* sp. strain CP4, which is widespread in the environment and can be found on plant produce, especially raw vegetables
- no sequence homology has been found between CP4 EPSPS and any known toxic or allergenic proteins
- the CP4 EPSPS protein is readily inactivated by heat and rapidly degraded by simulated mammalian digestive conditions.

82. Food derived from GM canola, cotton, lucerne, maize, soybean and sugarbeet crops that express the CP4 EPSPS protein have been considered safe for human consumption by FSANZ (ANZFA, 2000; FSANZ, 2005b, d, 2006a, b, 2007, 2013b).

5.1.6 Toxicity of herbicide metabolites

83. 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 MS11 × RF3, and glufosinate and glyphosate on MS11 × RF3 × MON 88302.

Glufosinate metabolites

84. 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 and DIR 175) and cotton (DIR 062/2005, DIR 143 and DIR 173). The main points are:

- Glufosinate causes plant cells to die by inhibiting the enzyme glutamine synthase, leading to accumulation of toxic levels of ammonia (OECD, 2002)
- The PAT enzyme, encoded by the *bar* gene, inactivates the L-isomer of glufosinate by acetylating it to *N*-acetyl-L-glufosinate (NAG), which does not inhibit glutamine synthase (Dröge et al., 1992; OECD, 2002)
- Following application of glufosinate to GM plants expressing PAT, the major residue present is NAG, with lower concentrations of glufosinate and 3-methyl phosphinico-propionic acid (MPP) (OECD, 2002)
- Following application of glufosinate to non-GM plants, the major residue is glufosinate, with a small proportion of MPP (OECD, 2002). *N*-acetyl-L-glufosinate is not present
- Both NAG and MPP are less toxic than glufosinate (FAO, 2014).

85. Recently, it was shown that PAT acetylates two plant endogenous amino acids, aminoadipate and tryptophan (Christ et al., 2017). Little safety data is available for *N*-acetyl-L-2-aminoadipate and *N*-acetyl-L-tryptophan; however, there is no suggestion that these metabolites are toxic to humans or animals at the levels present in GM canola (O'Connor, 2017).

Glyphosate metabolites

86. There is no expected difference in the metabolic fate of glyphosate in non-GM canola and in GM canola expressing the *cp4 epsps* gene. The CP4 EPSPS protein encoded by the *cp4 epsps* gene is naturally insensitive to the effects of glyphosate (Padgette et al., 1993), as are a number of other microbial EPSPS enzymes (Schulz et al., 1985; Eschenburg et al., 2002). Consequently, in GM plant cells with the *cp4 epsps* gene, biosynthesis of aromatic amino acids is not inhibited in the presence of glyphosate. Therefore, no new metabolic products are formed in these GM plants as the only difference from the native EPSPS enzyme is the reduced affinity for glyphosate (OECD, 1999a).

5.2 Toxicity/allergenicity of the parental GM canola lines

87. The Regulator concluded in the RARMPs for the parental GM canola lines that they are as safe as non-GM canola. A summary of this information including new or updated information since the original RARMPs is provided below.

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

5.2.1 Toxicity/allergenicity to humans

89. Canola oil is the only food product consumed by people, and oil from all GM parental lines has been approved for human consumption in Australia (ANZFA, 2001b; FSANZ, 2013b, 2017) and other countries (Section 3).

5.2.2 Toxicity to animals including livestock

90. Canola meal is produced as a by-product during the extraction of oil from canola seed. It is a significant component and a rich source of protein in livestock feed in Australia. Unprocessed canola seed can also be used directly as animal feed. In addition, canola can be used as a dual-purpose crop in Australia, whereby it is used for forage prior to seed production (Kirkegaard et al., 2008).

91. Toasted canola meal is the most common fraction used as animal feed, although some meal (20%) is physically extracted without added heat. A small amount (5%) of canola meal available in Australia is from cold-pressed seed (Mailer, 2004).

92. 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. Previous compositional analyses demonstrated that the levels of erucic acid and glucosinolates in MS11, RF3 and MON 88302 canola were below the industry standard of 30 µmoles of glucosinolates per g and do not vary significantly from their parental cultivars or other commercially available canola.

93. The parental GM canola lines are compositionally equivalent to non-GM canola varieties, with no meaningful differences other than the presence of the introduced proteins, and feeding studies on a range of organisms demonstrate that there are no anti-nutritional effects of the genetic modifications in the parental GM canola lines (ANZFA, 2001a; FSANZ, 2013b, 2017).

5.2.3 Toxicity to other organisms

94. A number of overseas regulatory agencies have assessed whether the parental GM canola lines have any increased toxicity to non-target organisms as a result of the genetic modifications. In its assessments of canola lines MS11, RF3 and MON 88302, the USDA-APHIS determined that the GM canola lines 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, 2013, 2017). The Canadian Food Inspection Agency (CFIA) concluded that the unconfined release of lines MS11, RF3 and MON 88302 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, 2012, 2018).

95. The Barnase and Barstar proteins are only expressed in the tapetal cell layer during anther development when the genes are controlled by the tapetal cell specific promoter PTa29. The Barstar protein expressed in MS11 plants is also at low level as the gene is controlled by the weak promoter PssuAt. Therefore, exposure to residues of these proteins from the GM plants from MS 11 and RF3 is expected to be low.

96. No significant differences were observed in a study evaluated in the DIR 127 RARMP, between MON 88302 canola and non-GM canola crops for the abundance of beneficial arthropods: chironomid midge, lacewings (Chrysopidae), ladybird beetles (Coccinellidae), micro- and macro-parasitic hymenoptera, miniature pirate bug (*Orius spp.*), spiders (Aranaea) and sphecid wasps (Sphecidae) (Monsanto Company, 2011).

5.3 Weediness of the parental GM canola lines

97. The weediness of the GM parental canola lines was assessed in the RARMPs for DIR 021/2002, DIR 127 and DIR 175 as posing negligible risk, and no credible reports of adverse outcomes as a result of the authorised releases have been received (Section 3).

98. Multiple herbicide tolerant individuals are as susceptible to alternative herbicides as single-herbicide tolerant canola plants or their non-GM counterparts (Beckie et al. 2004).

99. InVigor® canola hybrids based on the *barnase*/*barstar* hybrid breeding system have displayed yield increases of up to 22% over non-GM open pollinated varieties in Canada (Clayton et al., 1999; Zand and

Beckie, 2002; Harker et al., 2003). However, the superior seedling emergence and increased seed numbers (Clayton et al., 1999; Harker et al., 2003) does not lead to the expected increase in volunteers in commercial fields in Canada (Beckie and Owen, 2007) or in trials in the UK, due to greater uniformity in ripening (Crawley et al., 1993; Sweet, 1999; MacDonald and Kuntz, 2000). Data obtained in Australia indicate that the vigour exhibited by InVigor® canola hybrids falls within the range of vigour exhibited by non GM hybrid and open pollinated varieties of canola grown commercially (DIR 021/2002).

100. The Conservation Council of Western Australia published a survey of roadside canola plants conducted by the Conservation Council (WA) Citizen Science Program, Esperance Local Environmental Action Forum and GM Cropwatch. The survey was conducted in September 2011 to determine the frequency and distribution of GM Roundup Ready® canola plants in the Esperance region of WA after one year of commercial production. Among the 190 canola plants collected and tested, two GM positive plants were detected, representing about 1%. The area sown to GM canola was around 8% of the total canola crop in WA in 2010 (DAFWA, 2010).

Section 6 The GMOs proposed for release

6.1 Introduction to the GMOs

101. The GMOs proposed for release are GM lines MS11 × RF3 and MS11 × RF3 × MON 88302. MS11 × RF3 is derived from conventional breeding between the male sterile line MS11 and the fertility restoration line RF3, while MS11 × RF3 × MON 88302 is derived from conventional breeding between MS11 and RF3 and MON 88302.

102. The applicant has indicated that the intermediate parental lines MS11 x MON 88302 and RF3 x MON 88302, created through conventional breeding, would be part of the commercial release, as these would be used in the seed production process. Crossing between these lines would yield MS11 x RF3 x MON 88302. The applicant has also indicated that the two intermediate parental lines may potentially be sold as a commercial product, but it is expected the scale of cultivation of these two lines would be vastly less than to the two primary GM lines (MS11 × RF3 and MS11 × RF3 × MON 88302).

103. While the parental male sterile line MS11 and the intermediate parental line MS11 x MON 88302 used for breeding does not produce pollen, both of the GMOs are fully fertile.

104. The introduced genes in all four GM canola lines are shown in Table 6. Regulatory sequences for controlling the spatial expression of the genes will also be present (see Table 5 for details).

Table 6 The introduced genes present in the GM canola lines proposed for release

GM canola	Hybrid breeding system	Glufosinate tolerance	Glyphosate tolerance
MS11 × RF3	*barnase* *barstar* (3 copies)	*bar* (*2 copies*)	
MS11 × RF3 ×MON 88302	*barnase* *barstar* (3 copies)	*bar* (*2 copies*)	*cp4 epsps*
MS11 × MON 88302 (intermediate parent)	*barnase* *barstar* (not active in anthers)	*bar*	*cp4 epsps*
RF3 × MON 88302 (intermediate parent)	*barstar* (2 copies)	*bar*	*cp4 epsps*

6.2 Characterisation of the GMOs

6.2.1 Stability and molecular characterisation

105. Southern blot analysis was used to demonstrate the molecular equivalence of the MS11, RF3 and MON 88302 events in MS11 × RF3 and MS11 × RF3 × MON 88302 canola to the same events in the individual parental lines using event-specific T-DNA probes (Bayer, 2016c; BASF, 2018b). These results confirm the intactness of the GM loci and their flanking regions in the GM canola lines, indicating that no rearrangement occurred during conventional breeding.

6.2.2 Levels of the introduced proteins in the GM canola lines

106. The applicant has supplied two studies regarding the expression levels of the Barnase, Barstar, PAT proteins in MS11 × RF3 canola (Bayer, 2015) and these proteins plus the CP4 EPSPS protein in MS11 × RF3 × MON 88302 canola (BASF, 2018a). The expression levels of these proteins were determined by enzyme-linked immunosorbent assay (ELISA) in whole plant, root, raceme and grain tissues collected from plants treated and untreated with relevant herbicides.

MS11 × RF3

107. For the study on MS11 × RF3, plant tissue samples (both treated and untreated with glufosinate-ammonium) from MS11 × RF3, together with its parental lines MS11 and RF3, were collected from two sites in Canada and one site in the USA during the 2014 season. Levels of expressed proteins from the introduced genes in these GM canola lines were measured in plant tissues collected at growth stages of BBCH[3] 13-15 (3–5 leaf), BBCH 30-39 (stem elongation), BBCH 57-65 (first flowering) and BBCH 87-99 (maturity). Protein expression levels for tissues from herbicide treated plants are provided in Table 7. 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 across all sites (n=15), with some sample values excluded from calculations when values are below the lower limit of quantification (LLOQ) or not available for analysis.

Table 7 Expression levels of introduced proteins in MS11 × RF3 grown in Canada and the USA during 2014 (glufosinate treated)

Protein	Tissue (Growth stage)	Line		
		MS11× RF3	**MS11**	**RF3**
Barnase Mean ± SD (range) µg/g dw	Whole plant (BBCH 13-15)	ND (<LLOQ)	ND (<LLOQ)	ND (<LLOQ)
	Whole plant (BBCH 30-39)	ND (<LLOQ)	ND (<LLOQ)	ND (<LLOQ)
	Root (BBCH 30-39)	ND (<LLOQ)	ND (<LLOQ)	ND (<LLOQ)
	Whole plant (BBCH 57-65)	ND (<LLOQ)	ND (<LLOQ)	ND (<LLOQ)
	Root (BBCH 57-65)	4.74 ± ND (**n=1**) (<LLOQ – 4.74)	ND (<LLOQ)	ND (<LLOQ)
	Raceme (BBCH 57-65)	ND (<LLOQ)	ND (<LLOQ)	ND (<LLOQ)
	Grain (BBCH 87-99)	ND (<LLOQ)	ND (<LLOQ)	ND (<LLOQ)
Barstar Mean ± SD (range)	Whole plant (BBCH 13-15)	ND (<LLOQ)	ND (<LLOQ)	ND (<LLOQ)
	Whole plant	ND	ND	0.33 ± ND (**n=1**)

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

µg/g dw	(BBCH 30-39)	(<LLOQ)	(<LLOQ)	(<LLOQ – 0.33)
	Root (BBCH 30-39)	0.70 ± 0.21 (0.38 – 0.91)	0.50 ± 0.24 (0.27 – 1.04)	ND (<LLOQ)
	Whole plant (BBCH 57-65)	0.38 ± 0.07 (0.27 – 0.47)	0.21 ± 0.08 (0.13 – 0.28)	0.18 ± ND (**n=1**) (0.18 – 0.18)
	Root (BBCH 57-65)	0.45 ± 0.20 (0.28 – 0.85)	0.39 ± 0.10 (0.22 – 0.56)	ND (<LLOQ)
	Raceme (BBCH 57-65)	0.79 ± 0.94 (0.20 – 3.64)	0.68 ± 0.31 (0.46 – 0.90)	1.28 ± 1.01 (0.33 – 3.38)
	Grain (BBCH 87-99)	ND (<LLOQ)	ND (<LLOQ)	ND (<LLOQ)
PAT Mean ± SD (range) µg/g dw	Whole plant (BBCH 13-15)	52.09 ± 18.17 (20.42 – 83.02)	35.40 ± 16.22 (7.32 – 74.44)	63.71 ± 37.54 (23.91 – 181.94)
	Whole plant (BBCH 30-39)	25.52 ± 7.87 (12.98 – 37.60)	21.89 ± 9.59 (7.35 – 40.66)	56.84 ± 22.66 (28.11 – 107.38)
	Root (BBCH 30-39)	2.35 ± 4.80 (0.56 – 19.50)	0.39 ± 0.19 (0.18 – 0.64)	2.56 ± 2.33 (0.95 – 10.57)
	Whole plant (BBCH 57-65)	28.07 ± 13.10 (12.30 –59.38)	14.82 ± 5.0 (6.13 – 27.52)	43.20 ± 20.16 (6.49 – 89.33)
	Root (BBCH 57-65)	0.75 ± 0.36 (0.30 – 1.49)	0.37 ± 0.25 (0.15 – 0.76)	1.62 ± 0.85 (0.36 – 3.53)
	Raceme (BBCH 57-65)	41.94 ± 20.83 (17.04 – 108.12)	23.89 ± 10.73 (9.37 – 55.29)	40.59 ± 13.29 (12.54 – 62.63)
	Grain (BBCH 87-99)	0.60 ± 0.30 (0.25 – 1.12)	0.49 ± 0.18 (0.31 – 0.84)	0.83 ± 0.25 (0.57 – 1.39)

dw, dry weight; LLOQ, lower limit of quantification; ND, not determined; SD, standard deviation

108. As shown in Table 7, the level of Barnase expression in MS11 × RF3 in all tissue samples was below LLOQ, except for one root sample at first flowering stage (BBCH 57-65). The Barnase protein level in this root sample was 4.74 µg/g dw. Two root samples at the same growth stage from untreated plants also showed a mean protein level of 2.18 µg/g DW (details not shown). However, these protein expression levels were close to LLOQ when calculated on a fresh weight basis. Overall, the Barnase expression in MS11 × RF3 was considered consistent with the parental line MS11, which showed Barnase expression levels in all tissue samples below LLOQ.

109. Expression of Barstar in MS11 × RF3 was confirmed to exhibit a very similar pattern to that of MS11, primarily because expression of one of the two *barstar* genes is controlled by the weak constitutive Pnos promoter (Section 5.1.2). For both MS11 × RF3 and MS11, low levels of Barstar were expressed in all root tissues and whole plant at stem elongation and first flowering stages (including raceme), while young plants and grain showed Barstar expression at levels below LLOQ. This is in contrast with RF3, which only displayed a higher level of Bastar expression in the raceme (mean value of 1.28 µg/g dw), as its *barstar* gene is controlled by the tapetum-specific PTa29 promoter (Section 5.1.2).

110. Expression of PAT was measurable in all sampled plant tissues in MS11 × RF3. The mean PAT protein levels in all plant tissues from MS11 × RF3 were generally similar to that from RF3 but higher than that from MS11. Therefore, although MS11 × RF3 contains two copies of the *bar* gene, no obvious enhanced PAT expression levels were observed compared to RF3. The mean PAT protein level in MS11 × RF3 was highest in whole plant at 3-5 leaf stage and lowest in grain.

Table 8 Expression levels of introduced proteins in MS11× RF3 × MON 88302 grown in Canada and the USA during 2017 (glufosinate and glyphosate treated)

Protein	Tissue (Growth stage)	Line			
		MS11× RF3 × MON 88302	**MS11**	**RF3**	**MON 88302**
Barnase Mean ± SD (range) µg/g dw	Whole plant (BBCH 13-15)	ND (<LLOQ)	ND (<LLOQ)	N/A	N/A
	Whole plant (BBCH 30-39)	ND (<LLOQ)	ND (<LLOQ)	N/A	N/A
	Root	ND	ND	N/A	N/A

Protein	Tissue (Growth stage)	Line			
		MS11× RF3 × MON 88302	MS11	RF3	MON 88302
	(BBCH 30-39)	(<LLOQ)	(<LLOQ)		
	Whole plant (BBCH 57-65)	ND (<LLOQ)	ND (<LLOQ)	N/A	N/A
	Root (BBCH 57-65)	ND (<LLOQ)	ND (<LLOQ)	N/A	N/A
	Raceme (BBCH 57-65)	0.38 ± ND (<LLOQ – 0.38)	ND (<LLOQ)	N/A	N/A
	Grain (BBCH 87-99)	ND (<LLOQ)	ND (<LLOQ)	N/A	N/A
Barstar Mean ± SD (range) µg/g dw	Whole plant (BBCH 13-15)	ND (<LLOQ)	ND (<LLOQ)	ND (<LLOQ)	N/A
	Whole plant (BBCH 30-39)	0.26 ± ND (<LLOQ – 0.26)	ND (<LLOQ)	0.24 ± ND (<LLOQ – 0.24)	N/A
	Root (BBCH 30-39)	0.78 ± ND (<LLOQ – 0.78)	1.39 ± ND (<LLOQ – 1.39)	ND (<LLOQ)	N/A
	Whole plant (BBCH 57-65)	0.33 ± ND (<LLOQ – 0.33)	0.25 ± ND (<LLOQ – 0.25)	0.59 ± ND (0.18 – 0.59)	N/A
	Root (BBCH 57-65)	ND (<LLOQ)	1.01 ± ND (<LLOQ – 1.01)	ND (<LLOQ)	N/A
	Raceme (BBCH 57-65)	1.72 ± ND (<LLOQ – 1.72)	0.46 ± ND (<LLOQ – 0.46)	2.18 ± ND (<LLOQ – 2.18)	N/A
	Grain (BBCH 87-99)	ND (<LLOQ)	ND (<LLOQ)	ND (<LLOQ)	N/A
PAT Mean ± SD (range) µg/g dw	Whole plant (BBCH 13-15)	62.16 ± 31.45 (28.22 – 141.66)	44.63 ± 27.06 (22.27 – 118.72)	83.42 ± 30.13 (34.74 – 129.67)	N/A
	Whole plant (BBCH 30-39)	29.04 ± 8.41 (13.48 – 46.66)	28.75 ± 23.31 (<LLOQ – 73.35)	59.81 ± 18.95 (23.03 – 89.87)	N/A
	Root (BBCH 30-39)	3.86 ± 2.93 (0.98 – 8.80)	2.05 ± 3.15 (<LLOQ – 10.81)	5.19 ± 2.78 (2.11 – 11.54)	N/A
	Whole plant (BBCH 57-65)	41.69 ± 21.84 (<LLOQ –70.80)	29.25 ± 18.39 (<LLOQ – 57,98)	65.60 ± 15.44 (43.90 – 99.42)	N/A
	Root (BBCH 57-65)	1.78 ± 1.65 (0.61 – 5.75)	1.58 ± 1.87 (<LLOQ – 4.52)	2.67 ± 1.98 (0.54 – 7.18)	N/A
	Raceme (BBCH 57-65)	50.60 ± 21.41 (23.66 – 88.49)	24.83 ± 22.29 (<LLOQ – 70.40)	71.69 ± 37.32 (<LLOQ – 132.92)	N/A
	Grain (BBCH 87-99)	0.97 ± 0.29 (0.53 – 1.26)	0.51 ± 0.07 (0.39 – 0.61)	0.94 ± 0.20 (0487 – 1.22)	N/A
CP4 EPSPS Mean ± SD (range) µg/g dw	Whole plant (BBCH 13-15)	103.03 ± 26.10 (42.44 – 141.27)	N/A	N/A	169.47 ± 61.9 (67.12 – 276.76)
	Whole plant (BBCH 30-39)	66.34 ± 15.55 (45.71 – 92.28)	N/A	N/A	133.8 ± 31.45 (69.86 – 169.24)
	Root (BBCH 30-39)	47.78 ± 15.16 (25.51 – 65.67)	N/A	N/A	93.05 ± 23.59 (57.72 – 135.21)
	Whole plant (BBCH 57-65)	111.65 ± 43.55 (36.31 – 174.50)	N/A	N/A	224.03 ± 55.19 (135.12 – 318.66)
	Root (BBCH 57-65)	35.67 ± 7.58 (26.07 – 49.01)	N/A	N/A	81.64 ± 27.49 (48.32 – 130.97)
	Raceme (BBCH 57-65)	135.51 ± 62.44 (75.90 – 302.28)	N/A	N/A	323.31 ± 256.94 (110.01 – 1060.51)
	Grain (BBCH 87-99)	17.22 ± 1.65 (14.73 – 19.84)	N/A	N/A	27.71 ± 3.77 (23.14 – 34.95)

dw, dry weight; LLOQ, lower limit of quantification; ND, not determined; N/A, not applicable; SD, standard deviation

MS11 × RF3 × MON 88302

111. Plant tissue samples from MS11 × RF3 × MON 88302 (treated and untreated with glufosinate-ammonium and glyphosate), together with its parental lines MS11 and RF3 (treated and untreated with glufosinate-ammonium) and MON 88302 (treated and untreated with glyphosate), were collected from two sites in Canada and one site in the USA during the 2017 season. Levels of expressed proteins from

the introduced genes in these GM canola lines were measured in plant tissues collected at the same growth stages as for the above study on MS11 × RF3. Protein expression levels for tissues from herbicide treated plants are provided in Table 8.

112. As shown in Table 8, the level of Barnase expression in MS11 × RF3 × MON 88302 in all tissue samples was below LLOQ, except for one raceme sample at first flowering stage (BBCH 57-65). The Barnase protein level in this raceme sample was 0.38 µg/g dw, which is close to LLOQ when calculated on a fresh weight basis. Overall, the Barnase expression in MS11 × RF3 × MON 88302 was considered consistent with MS11, which showed Barnase expression levels in all tissue samples below LLOQ.

113. MS11 × RF3 × MON 88302 showed very similar patterns and levels of Barstar and PAT expression levels in all sampled tissues as MS11 × RF3.

114. Expression of CP4 EPSPS was measurable in all sampled plant tissues in MS11 × RF3 × MON 88302. The mean CP4 EPSPS protein levels in all plant tissues from MS11 × RF3 × MON 88302 were almost only half of that from its parental line MON 88302. The mean CP4 EPSPS protein level in MS11 × RF3 × MON 88302 was highest in raceme and lowest in grain.

6.2.3 Phenotypic characterisation and environmental interaction

115. Phenotypic characterisation (including agronomic characters) and environmental interaction data for MS11 × RF3 and MS11 × RF3 × MON 88302 were collected from field trials conducted in canola growing regions in Canada and the USA during 2014 (Bayer, 2016a) and 2017 (BASF, 2019b), respectively. In each study, ten trial sites were selected that provided a range of environmental and agronomic conditions representative of those 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. Australian canola growing areas 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 (eg temperate) are climatically similar to those of the selected trial sites in Canada and the USA. In addition, canola is a crop plant species with a long 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). These studies are therefore relevant to the Australian environment (Fischer et al., 2021). The varied environmental and agronomic conditions of the selected trial sites enable comparison of the GM canola lines with their non-GM parental canola and other conventional canola varieties under similar climatic conditions experienced in Australia.

116. Both trait-specific herbicide treated and untreated MS11 × RF3 (glufosinate) and MS11 × RF3 × MON 88302 (glufosinate and glyphosate) plants are included in the 2014 study and 2017 study, respectively. As the parental canola line for generating the MS11 canola is N90-740 and MS11 is the maternal line for producing the hybrid lines MS11 × RF3 and MS11 × RF3 × MON 88302, the canola line N90-740 was also included in the studies as a control for the conventional counterpart. Six additional non-GM commercial canola varieties were also included in each study as reference varieties to generate reference ranges for agronomic parameters for comparison. The reference range for each measured phenotypic characteristic was determined from the minimum and maximum mean values from the six reference canola varieties planted among the sites. Comparisons of MS11 × RF3/MS11 × RF3 × MON 88302 and the control N90-740 were conducted within each site (individual site analysis) and in a combined-site analysis, in which the data were pooled across sites for phenotypic characteristics. Data presented in Tables 9 – 11 are from combined-site analysis and numbers represent sample means with standard deviation (SD). Statistical differences were identified at a 5% level of significance ($p<0.05$).

Phenotypic and agronomic characterisation

MS11 × RF3

117. In the 2014 study (Bayer, 2016a), the phenotypic and agronomic characteristics measured include early and final stand count, days to flowering, days to 10% plants remaining at flowering, days to maturity, yield, plant height, seedling vigor, plant lodging and pod shattering. Combined-site comparison of these agronomic parameters from herbicide treated MS11 × RF3 plants and the control N90-740 is provided in Table 9. There were no statistically significant differences observed between herbicide treated MS11 × RF3 and the control N90-740 canola for any of these agronomic parameters. This is also the same for MS11 × RF3 not treated with herbicide (data not shown). All values of these measured agronomic parameters for MS11 × RF3 were also within the range of the reference varieties. This indicates that MS11 × RF3 has no biologically relevant differences for the measured agronomic characteristics compared to conventional canola varieties.

Table 9 Combined-site analysis of agronomic parameters of MS11 × RF3 (herbicide treated) and the control N90-740 canola across all sites from the field trials in Canada and the USA during 2014

Parameter	MS11 × RF3 Mean ± SD	Control Mean ± SD	Reference range	p-value
Early stand count	150.9 ± 49.3	158.3 ± 49.2	65 - 312	0.341
Final stand count	98.7 ± 38.7	95.9 ± 32.4	15 - 174	0.604
Days to flowering	43.6 ± 4.2	43.5 ± 4.1	37 - 55	0.930
Days to flowering - 10% remains	61.5 ± 8.3	61.8 ± 8.7	46 - 76	0.738
Days to maturity	100.8 ± 10.7	100.5 ± 10.1	80 - 125	0.725
Average plant height (cm)	109.1 ± 21.4	112.7 ± 22.7)	76.8 - 154.5	0.164
Yield (Kg/Ha)	1719.4 ± 929.3	1638.0 ± 959.5	241.0 - 3760.4	0.449
Seeding vigor (1-9)	7.0 ± 1.7	6.8 ± 1.7	1 - 9	0.579
Lodged plants (1-9)	5.7 ± 2.2	5.5 ± 2.2	1 - 9	0.585
Pod shattering (1-9)	8.0 ± 1.1	8.0 ± 1.1	4 - 9	0.577

MS11 × RF3 × MON 88302

118. In the 2017 study (BASF, 2019b), the agronomic characteristics measured include early stand count, crop development, days to flowering, flowering duration, final stand count, plant height, days to maturity, lodging, fruit count, seed loss, seed yield and thousand seed weight. Combined-site comparison of these agronomic parameters from herbicide treated MS11 × RF3 × MON 88302 plants and the control N90-740 canola is provided in Table 10.

119. There were no statistically significant differences detected between herbicide treated MS11 × RF3 × MON 88302 and the control N90-740 canola for crop development, days to flowering, days to maturity, fruit count and seed loss. Statistically significant differences ($p < 0.05$) were observed between herbicide treated MS11 x RF3 x MON 88302 and the control N90-740 canola for early stand count, flowering duration, final stand count, plant height, lodging, seed yield and thousand seed weight. Statistically significant differences were also observed for MS11 x RF3 x MON 88302 not treated with herbicide for

final stand count, plant height, lodging and seed yield (data not shown). However, the mean values of these agronomic parameters displaying statistically significant differences for MS11 x RF3 x MON 88302 were all within the range of the reference varieties, indicating that MS11 x RF3 x MON 88302 has no biologically meaningful agronomic differences to conventional canola varieties.

Table 10 Combined-site analysis of agronomic parameters of MS11 × RF3 × MON 88302 (herbicide treated) and the control N90-740 canola across all sites from the field trials in Canada and the USA during 2017

Parameter	MS11 × RF3× MON 88302 Mean ± SD	Control Mean ± SD	Reference range	p-value
Early stand count	183.27 ± 67.86	160.61 ± 80.21	11.67 – 387.04	0.004
Crop development (%)	90.3 ± 10.4	85.0 ± 12.1	40 – 100	0.056
Days to flowering	41.0 ± 1.8	40.8 ± 2.0	36 – 49	0.690
Flowering duration	61.2 ± 9.0	59.2 ± 8.1	45 – 88	0.035
Final stand count	132.88 ± 39.62	108.47 ± 41.74	28.89 – 275.93	0.001
Plant height (cm)	114.7 ± 15.0	103.5 ± 12.9	78.8 – 142.2	<.001
Days to maturity	90.1 ± 6.3	88.3 ± 6.3	78 – 102	0.075
Lodging (%)	16.3 ± 19.4	29.8 ± 26.6	0 – 90	0.038
Fruit count	115.5 ± 92.9	102.7 ± 71.8	29 – 416	0.095
Seed loss	7.98 ± 15.93	10.98 ± 16.02	0 – 116	0.072
Seed yield (T/Ha)	2.22 ± 0.85	1.42 ± 0.78	0.334 – 4.206	<.001
1000 seed weight (g)	4.00 ± 0.83	3.72 ± 0.65	2.43 – 6.59	0.014

Environmental interaction

120. Environmental interaction refers to the interaction between the crop plants and their receiving environment. The environmental interaction data collected included plant response to abiotic stressors, disease and insect damage. At least three abiotic stressors, three diseases and three insect pests were evaluated at four intervals during the growing season. The four intervals were the growth stages at leaf development, stem elongation, flowering and pod development.

MS11 × RF3

121. In the 2014 study (Bayer, 2016a), plant response to abiotic stress, disease damage and arthropod damage was quantitatively assessed. Combined-site comparison of these abiotic and biotic stress parameters from herbicide treated MS11 × RF3 plants and the control N90-740 canola is provided in Table 11.

Table 11 Environmental interaction of MS11 × RF3 (herbicide treated) and the control N90-740 canola across all sites from the field trials in Canada and the USA during 2014

Parameter (Stress rating 1-9*)	MS11 × RF3 Mean ± SD	Control Mean ± SD	Reference range	p-value
Abiotic Stress (BBCH 12-14)	1.0 ± 0.2	2.2 ± 1.9	1 - 9	0.415
Abiotic Stress (BBCH 30-39)	1.9 ± 1.2	1.7 ± 1.0	1 - 4	0.150
Abiotic Stress (BBCH 60-69)	1.5 ± 1.0	1.4 ± 0.9	1 - 7	0.719
Abiotic Stress (BBCH 79-87)	3.0 ± 2.2	3.1 ± 2.2	1 - 7	0.247
Disease Stress (BBCH 12-14)	1.0 ± 0.2	1.1 ± 0.5	1 - 3	0.111
Disease Stress (BBCH 30-39)	1.0 ± 0.0	1.0 ± 0.0	1 - 1	NA
Disease Stress (BBCH 60-69)	1.3 ± 0.7	1.3 ± 0.7	1 - 3	1.000
Disease Stress (BBCH 79-87)	1.9 ± 1.2	1.9 ± 1.1	1 - 5	0.544
Insect Stress (BBCH 12-14)	1.1 ± 0.3	1.2 ± 0.5	1 - 3	0.259
Insect Stress (BBCH 30-39)	1.5 ± 1.0	1.6 ± 1.0	1 - 4	0.711
Insect Stress (BBCH 60-69)	1.8 ± 1.3	1.7 ± 1.4	1 - 5	0.659
Insect Stress (BBCH 79-87)	1.3 ± 0.8	1.4 ± 0.8	1 - 3	0.495

* Stress rating 1 = Little to no stressor present, 3 = Stressor present but symptoms are light or patchy and effect on yield and plant growth are likely negligible, 5 = Stressor symptoms apparent and more consistent through the plot; more obvious that external Stressors are at play; effects on yield and plant growth somewhat uncertain but certainly possible, 7 = Stressor symptoms are obvious; likely to affect yield/quality, 9 = Stressor symptoms are severe; crop damage and yield loss are certain and significant; NA, no analysis due to lack of variability

122. There were no statistically significant differences detected between herbicide treated MS11 × RF3 and the control N90-740 canola for all measured abiotic and biotic stress parameters. All mean values of these measured environmental stress parameters are within the range of the reference varieties. The same observations were made for MS11 × RF3 not treated with herbicide (data not shown). Therefore, MS11 × RF3 is considered comparable to conventional canola in its response to environmental stresses.

MS11 × RF3 × MON 88302

123. In the 2017 study (BASF, 2019b), plant response to abiotic stress, disease damage and arthropod damage was qualitatively assessed. The abiotic stressors, diseases and pest arthropods selected for this assessment were: abiotic stressors – cold, drought, flood, frost, hail, heat, nutrient deficiency, soil compaction, soil crusting, wet soil and wind damage; diseases – Alternaria black spot, anthracnose, Aster yellows, black leg, Cercospora leaf spot, clubroot, Downey mildew, Fusarium wilt, gray mold, powdery mildew, Phytophthora root rot, Pythium, Rhizoctonia, root maggots, Sclerotinia, seedling disease complex, and wirestem; and arthropods – 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, slug, swede midge, and thrips. A total of 120 valid comparisons between MS11 x RF3 x MON 88302 and the control N90-740 canola were carried out for each of the categories for abiotic stressor, disease damage and arthropod damage. No meaningful

differences were observed between MS11 x RF3 x MON 88302 and the control for any of these comparisons among all observations at the sites.

6.2.4 Compositional analysis

124. The applicant provided data for compositional analysis of MS11 x RF3 and MS11 x RF3 x MON 88302 seed harvested from ten field trial sites in canola growing regions in Canada and the USA during 2014 (Bayer, 2016b) and 2017 (BASF, 2019a), respectively, in comparison to the canola line N90-740 (as control) and six reference non-GM commercial canola varieties. In both studies, each entry was replicated four times in a randomised complete block design at each field trial.

125. Both trait-specific herbicide treated and untreated MS11 × RF3 (glufosinate) and MS11 × RF3 × MON 88302 (glufosinate and glyphosate) plants are included in the 2014 study and 2017 study, respectively. Composition analyses were conducted to determine levels of nutrients and anti-nutrients in grain from MS11 × RF3 or MS11 x RF3 x MON 88302, the control N90-740 canola, and six non-GM reference canola varieties. Compositional data from non-GM commercial varieties grown concurrently in the same trial with MS11 × RF3 or MS11 x RF3 x MON 88302 and the control, were combined across all sites and used to calculate a 99% tolerance interval for each component to define the natural variability in commercial varieties. Any statistically significant differences ($p<0.05$) between MS11 × RF3 or MS11 x RF3 x MON 88302 and the control were also compared to this tolerance range, to assess whether the differences were likely to be biologically meaningful.

126. Analytes with more than one third of sample values below the limit of quantification (LOQ) were excluded from statistical analysis. Only statistical data for herbicide treated MS11 × RF3 or MS11 x RF3 x MON 88302 are discussed here as these canola lines are expected to be sprayed with trait-specific herbicide(s) under the commercial production conditions.

<u>MS11 × RF3</u>

127. In the 2014 study (Bayer, 2016b), grain samples from nine field trial sites were analysed for analytes including proximates, fibre, amino acids, fatty acids, minerals, vitamins, and anti-nutrients. A total of 92 analytes were measured, but 35 of the analytes were not statistically analysed as more than one third of sample values for these analytes were below LOQ. These include 23 fatty acids, one mineral (sodium), one type of vitamin E (β-tocopherol) and 10 anti-nutrient glucosinolates. The remaining 57 analytes (47 nutrients and ten anti-nutrients) were statistically assessed.

128. In the combined-site analysis, 30 of the 47 nutrient analytes showed no statistically significant difference between MS11 × RF3 and the control N90-740 canola. These are: two proximates, one type of fibre, 11 amino acids, eight fatty acids, six minerals and two types of vitamin E.

129. Statistically significant differences were identified in the other 17 nutrient analytes, with MS11 × RF3 having statistically significant increase ($p<0.05$) in one proximate (protein), one type of fibre (acid detergent fibre) and seven amino acids (arginine, cystine, glutamic acid, histidine, lysine, methionine, and proline), and statistically significant decrease ($p<0.05$) in two proximates (moisture and total carbohydrates), three fatty acids (palmitoleic, stearic and arachidic), two minerals (calcium and potassium) and one vitamin (vitamin K). However, all these nutrient mean values were within the range of the reference varieties and the 99% tolerance intervals established by the non-GM reference varieties grown concurrently in the same trials.

130. Among the anti-nutrients, no statistically significant differences between MS11 × RF3 and the control were identified in the combined-site analysis for 4-hydroxyglucobrassicin, glucobrassicin, gluconapin, progoitrin, total glucosinolates, phytic acid, insoluble tannins, soluble tannins and total condensed tannins. Statistically significant difference ($p<0.05$) was identified for increased level of sinapine. However, this anti-nutrient mean value was within the range of the reference varieties and the 99% tolerance interval established by the non-GM reference varieties.

131. In summary, seed from the MS11 × RF3 line is compositionally comparable with seed from non-GM canola varieties and the observed differences in the seed component values between MS11 × RF3 and the control N90-740 canola are not considered biologically meaningful from a food and feed perspective.

MS11 × RF3 × MON 88302

132. In the 2017 study (BASF, 2019a), grain samples from eight field trial sites were analysed for analytes including proximates, fibre, amino acids, fatty acids, minerals, vitamins, and anti-nutrients. A total of 94 analytes were measured but 36 of the analytes were not statistically analysed as more than one third of sample values for these analytes were below LOQ. These include 23 fatty acids, one mineral (sodium), two types of vitamin E (β-tocopherol and δ- tocopherol) and 10 anti-nutrient glucosinolates. The remaining 58 analytes (48 nutrients and ten anti-nutrients) were statistically assessed.

133. In the combined-site analysis, 19 of the 48 nutrient analytes showed no statistically significant difference between MS11 × RF3 x MON 88302 and the control N90-740 canola. These are: three proximates, two types of fibre, four amino acids, five fatty acids, three minerals and two types of vitamins.

134. Statistically significant differences were identified in the other 29 nutrient analytes, with MS11 × RF3 x MON 88302 having statistically significant increase ($p<0.05$) in two proximates (ash and crude protein), 14 amino acids, one fatty acid (oleic) and five minerals and statistically significant decrease ($p<0.05$) in five fatty acids and two types of vitamin E. However, all these nutrient mean values were within the range of the reference varieties and the 99% tolerance intervals established by the non-GM reference varieties grown concurrently in the same trials.

135. Among the anti-nutrients, no statistically significant differences between MS11 × RF3 and the control were identified in the combined-site analysis for 4-hydroxyglucobrassicin, progoitrin, total glucosinolates, sinapine and soluble tannins. Statistically significant difference ($p<0.05$) was identified for increased level of glucobrassicin, gluconapin, phytic acid and statistically significant decrease ($p<0.05$) in insoluble tannins and total tannins. However, these anti-nutrient mean values were within the range of the reference varieties and the 99% tolerance interval established by the non-GM reference varieties.

136. In summary, seed from the MS11 × RF3 x MON 88302 line is compositionally comparable with seed from non-GM canola varieties and the observed differences in the seed component values between MS11 × RF3 x MON 88302 and the control N90-740 canola are not considered biologically meaningful from a food and feed perspective.

Section 7 The receiving environment

137. 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).

138. The applicant has proposed to release the GM canola lines in all commercial canola growing areas, Australia-wide. Therefore, for this licence application, it is considered that the receiving environment is all of Australia, but in particular agricultural areas that are suitable to cultivate canola. Commercial canola production occurs mainly in WA, NSW, Victoria and SA, with small areas[4] grown in Queensland

[4] On average, a total of 1000 hectares in each state.

and Tasmania (ABARES, 2020). 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

139. In Australia, canola is commonly grown in rotation with cereal crops (OGTR, 2017). Canola is usually grown as a winter annual crop, with planting occurring in April or May and harvest in early summer so that yield is not affected by frost damage or hot, dry conditions (GRDC, 2015a, b, 2017a). Some late-maturing varieties can be grazed by livestock during winter, before plants are allowed to mature and set seed. Small areas of canola are also sown in late spring/early summer and harvested in early autumn in cool regions with high water availability. Canola has higher requirements for nitrogen, phosphorous and sulfur than most other crops so fertiliser application is important. Canola is harvested either by windrowing (swathing) or by direct harvesting. During windrowing, the crop is cut and gathered on top of the stubble into a pile, ideally 1.5 m wide and 1 m high (GRDC, 2009). After 1–2 weeks, when most of the seed has matured and the moisture content is under 9%, the windrow is picked up and threshed by the harvester. Standard cultivation practices for canola are discussed in more detail in *The Biology of* Brassica napus *L. (canola) and* Brassica juncea *(L.) Czern. & Coss. (Indian mustard)* (OGTR, 2017) and *Canola best practice management guide for south-eastern Australia* (GRDC, 2009).

140. It is anticipated that agronomic practices for the cultivation of the GM canola lines proposed for release would not differ from standard industry practices. Glufosinate and/or glyphosate may be applied over the top of the GM 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.

141. Crop Management Plans (CMPs) have been developed separately for MS11 × RF3 and MS11 × RF3 × MON 88302 canola that farmers growing the GM canola lines would be required to follow.

7.2 Relevant abiotic factors

142. The geographical distribution of commercial canola cultivation in Australia is limited by a number of abiotic factors, the most important being water availability. Canola is generally grown as a winter crop in winter-dominant medium and high rainfall environments that receive more than 350 mm rainfall per year (GRDC, 2009; OGTR, 2017). It can be grown in lower-rainfall zones as an opportunistic crop when there is good subsoil moisture, or at low plant population densities to reduce water requirements. Germination of seed will only occur if there is sufficient soil moisture, and drought stress after anthesis can significantly reduce yield due to abortion of seed and reduced pod numbers. Canola is also sensitive to waterlogging (GRDC, 2009; OGTR, 2017).

143. Other abiotic stresses that can reduce canola yields include frost, particularly during early pod development, and heat stress (GRDC, 2009). Additional information regarding abiotic factors relating to the growth and distribution of commercial canola in Australia is discussed in the reference document, *The Biology of* Brassica napus *L. (canola) and* Brassica juncea *(L.) Czern. & Coss. (Indian mustard)* (OGTR, 2017).

7.3 Relevant biotic factors

7.3.1 Presence of sexually compatible plants in the receiving environment

144. Gene transfer to sexually compatible plants in the receiving environment can occur via cross-pollination. Canola pollination is described in Section 4.

145. 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. For instance, of the 55 canola varieties

available for sale in NSW in 2020, approximately 35% are non-GM triazine tolerant (TT), 24% non-GM imidazolinone tolerant (IMI; Clearfield®), 27% GM glyphosate tolerant (GT; Roundup Ready® + TruFlex™ Roundup Ready®) and 7% conventional non-herbicide tolerant canola varieties (Matthews et al., 2020). Stacked varieties containing two herbicide tolerance traits (TT + IMI, TT + GT, IMI + GT) (7%), have also become available (Shackley et al., 2019; Matthews et al., 2020). The Clearfield® trait has also been available in *B. juncea* (Indian mustard or juncea canola) (GRDC, 2017a). The majority of canola varieties are hybrids, with only TT and conventional canola available as open pollinated varieties (Shackley et al., 2019; Matthews et al., 2020).

146. The GM canola varieties approved for commercial cultivation in Australia are listed in Table 12. MON 88302 (TruFlex™ Roundup Ready® canola), as a newer variant of Roundup Ready® canola, has become available to growers since 2019 (Shackley et al., 2019; Matthews et al., 2020). 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 2021 before becoming available to Australian growers in future (BASF website, accessed May 2021). MS8 × RF3 × MON 88302 (InVigor® x TruFlex™ Roundup Ready® 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 12 GM canola approved for commercial cultivation in Australia

DIR licence	Trade name	GM traits
020/2002	Roundup Ready® Canola	*cp4 epsps* and *goxv247*: tolerance to glyphosate herbicides
021/2002	InVigor® Canola	*barnase* and *barstar*: hybrid breeding system *bar* and *pat*: tolerance to glufosinate herbicides
108	InVigor® x Roundup Ready® Canola	*barnase* and *barstar*: hybrid breeding system *bar* and *pat*: tolerance to glufosinate herbicides *cp4 epsps* and *goxv247*: tolerance to glyphosate herbicides
127	TruFlex™ Roundup Ready® Canola	*cp4 epsps*: tolerance to glyphosate herbicides
138	InVigor® x TruFlex™ Roundup Ready® Canola	*barnase* and *barstar*: hybrid breeding system *bar*: tolerance to glufosinate herbicides *cp4 epsps*: tolerance to glyphosate herbicides
139	Optimum™ GLY Canola	*gat4621*: tolerance to glyphosate herbicides
155	N/A	Seven genes involved in metabolism of long-chain polyunsaturated fatty acids for omega-3 oil content *pat*: tolerance to glufosinate herbicides
175	N/A	*barnase* and *barstar*: hybrid breeding system *bar*: tolerance to glufosinate herbicides

147. Canola can cross with *B. napus* subspecies, including forage rape and vegetables such as swedes, if there is synchronicity of flowering. Brassica vegetables are generally harvested prior to flowering unless they are grown for seed production, in which case precautions would usually be taken to avoid crossing with canola (OGTR, 2017). Forage brassicas usually do not reach flowering due to re-sowing to new pastures or crops after grazing, and as flowering crops should not be fed to livestock (Harrington, 2012; Heritage Seeds, 2016).

148. *Brassica napus* (genome AACC) can also spontaneously cross with the related crop species *B. juncea* (AABB, including brown mustard) and *B. rapa* (AA, including turnips) (Warwick et al., 2003; Liu

et al., 2010; Liu et al., 2013), and there is one report of field crosses with the crop species *B. oleracea* (CC, including broccoli, cabbage, cauliflower and kale) (Ford et al., 2006).

149. Horticultural crops that are variants or subspecies of *B. juncea*, *B. rapa* or *B. oleracea* are commercially grown in Australia. *Brassica juncea* is grown in Australia as a broad-acre crop similar to canola, though at much smaller scale, and typically in low rainfall regions that are marginally suitable for canola (GRDC, 2017a). Recently, a forage brassica hybrid between *B. oleracea* and *Raphanus sativus* (RR, radish), known as a raphanobrassica (RRCC), has become available in Australia (PGG Wrightson Seeds, 2020).

150. Under open pollination conditions, naturally occurring hybrids between *B. napus* and the related weedy species *Raphanus raphanistrum* (genome RrRr, wild radish) and *Hirschfeldia incana* (AdAd, Buchan weed) have been reported at very low frequencies (Darmency et al., 1998; Darmency and Fleury, 2000). According to Weeds Australia (accessed April 2021), *R. raphanistrum* is a serious agricultural weed widespread throughout Queensland, NSW, Victoria, Tasmania, SA and WA. *Hirschfeldia incana* is a common roadside weed that is naturalised in Queensland, NSW, Victoria, Tasmania and SA, and can be problematic in winter cereal crops.

151. Naturally occurring hybrids between *B. napus* and *Sinapis arvensis* (genome SarSar, charlock or wild mustard) have been observed, but at an even lower frequency than hybrids with *R. raphanistrum* or *H. incana* (Lefol et al., 1996; Chevre et al., 2003). According to Groves et al. (2003), *S. arvensis* is primarily an agricultural or ruderal weed in Australia; however, it is not listed by Weeds Australia (accessed May 2021).

152. At the chromosomal level, gene transfer can occur between different sexually compatible species via recombination among homeologous chromosomes or via the creation of allopolyploids (Liu et al., 2013). A study of gene flow from *B. napus* to *R. raphanistrum*, in advanced generations of intergeneric hybrids, showed that regions of the *B. napus* A03 chromosome introgressed into *R. raphanistrum* chromosomes; however, the rate of gene flow from *B. napus* chromosome A03 was low compared with chromosomes A10 and C09 (Adamczyk-Chauvat et al., 2017).

7.3.2 Presence of related native plants in the receiving environment

153. 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).

154. Gene flow is less likely to occur between more distantly related species. The weedy genera discussed in the previous section (*Hirschfeldia*, *Raphanus* and *Sinapis*) belong to the tribe Brassiceae, along with the genus *Brassica* (Warwick et al., 2009). Thus, it is not plausible that gene flow could occur from *B. napus* to any native Australian plants under natural conditions.

7.3.3 Presence of other biotic factors

155. A number of diseases have the potential to significantly reduce the yield of canola. Blackleg disease caused by the fungal pathogen *Leptosphaeria maculans* is the most serious disease affecting commercial canola production in Australia (GRDC, 2009; OGTR, 2017). Blackleg is managed by choosing varieties with high blackleg resistance ratings and by planting canola at least 500 m from the previous year's stubble, which carries blackleg spores. Other damaging diseases of canola include stem rot caused by the fungus *Sclerotinia sclerotiorum* and damping-off, caused mainly by the fungus *Rhizoctonia solani* (GRDC, 2009).

156. Canola is most susceptible to insect pests during establishment of the crop, particularly from redlegged earth mites, blue oat mites, lucerne fleas, cutworms and aphids (as viral vectors) (GRDC,

2009). From flowering to crop maturity, severe damage can be caused by aphids, Rutherglen bugs, diamondback moth caterpillars and heliothis caterpillars.

157. Canola is 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 and glyphosate herbicides

158. There is potential for development of herbicide-resistant weeds if glufosinate and glyphosate herbicides are inappropriately used with MS11 × RF3 or MS11 × RF3 × MON 88302 canola. The repetitive use of a single herbicide, or herbicide group[5], 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).

159. 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.

160. Weeds resistant to glufosinate herbicides have been reported overseas; however, no glufosinate-resistant weed species have been reported in Australia (Heap, 2020). The species that are currently known to have developed resistance to glufosinate are goosegrass (*Eleusine indica*; Malaysia), Italian ryegrass (*Lolium multiflorum*; NZ, USA), perennial ryegrass (*L. perenne*; NZ), and rigid ryegrass (*L. rigidum*, annual ryegrass[6]; Greece).

161. Weeds resistant to glyphosate herbicides are more widely present. At least 53 weed species from around the world are reported to have resistance to glyphosate with 21 of them also found in Australia (Heap, 2020). According to a list of herbicide resistant weeds in Australia prepared by CropLife Australia (accessed May 2021), the most commonly found glyphosate-resistant weeds include annual bluegrass (*Poa annua*), annual ryegrass (*L. rigidum*), annual sowthistle *(Sonchus oleraceus*), awnless barnyard grass (*Echinochloa colona*), feather fingergrass (*Chloris virgata*), hairy fleabane (*Conyza bonariensis*), tall fleabane (*Conyza sumatrensis*), liverseedgrass (*Urochloa panicoides*) and windmill grass (*Chloris truncata*).

162. Stewardship guides and CMPs are prepared by companies selling herbicide tolerant canola seed, e.g. Advanta Seeds (2019), GenTech Seeds (2019). These guides are to be followed when growing herbicide tolerant varieties in order 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 MS11 canola and its commercial hybrid progeny with RF3 and MON 88302 canola. This will include the measures the same as those taken to manage volunteers in InVigor® × MON 88302 canola (DIR 138). The guidelines include good farm hygiene to minimise the occurrence of off-types and volunteers during production, handling, transport and storage or GM and non-GM canola.

[5] 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.

[6] In Australia, '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

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

164. 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, 1999b). Genes encoding PAT and similar acetyltransferase enzymes are present in a range of common soil bacteria, and are not known to be toxic or allergenic (Hérouet et al., 2005).

165. The bacterium *B. amyloliquefaciens*, from which the *barnase* and *barstar* genes were 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.

166. Barnase is a ribonuclease enzyme that is secreted by *B. amyloliquefaciens* into the soil and Barstar is a ribonuclease inhibitor protein, which specifically inhibits Barnase enzyme function. Nuclease enzymes and inhibitor proteins are ubiquitous in nature and can be found in plants, animals and microorganisms. Barnase is related to other ribonucleases, including ribotoxins and bacteriocins, found in bacteria and fungi (Yang, 2011). 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 classes of protein encoded by the introduced genes (ribonuclease and ribonuclease inhibitor) would be commonly encountered by other organisms in the environment.

167. The introduced *cp4 epsps* gene was isolated from the CP4 strain of the common soil bacterium *Agrobacterium* sp. The CP4 EPSPS protein is produced naturally by this strain (Padgette et al., 1995). This bacterium can also be found on plants and fresh plant produce. Genes coding for closely related EPSPS proteins are present in plants, bacteria and fungi (Gasser et al., 1988). The CP4 EPSPS protein expressed in the GM canola plants is functionally equivalent to endogenous plant EPSPS with the exception that CP4 EPSPS is less sensitive to glyphosate inhibition (Franz et al., 1997). CP4 EPSPS protein is also expressed in commercial varieties of GM canola and cotton grown in Australia.

168. Short regulatory sequences are derived from the bacterium *A. tumefaciens,* the plants *A. thaliana* (thale cress), *N. tabacum* (tobacco) and *Pisum sativum* (pea) and the plant viruses CMV and FMV. Although *A. tumefaciens*, CMV and FMV are plant pathogens, and tobacco produces toxins and carcinogens, the regulatory sequences comprise a small part of their total genome, and in themselves have no pathogenic, toxic or carcinogenic properties. With the exception of tobacco, which is no longer grown commercially in Australia, all the source organisms for the introduced 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

169. 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.

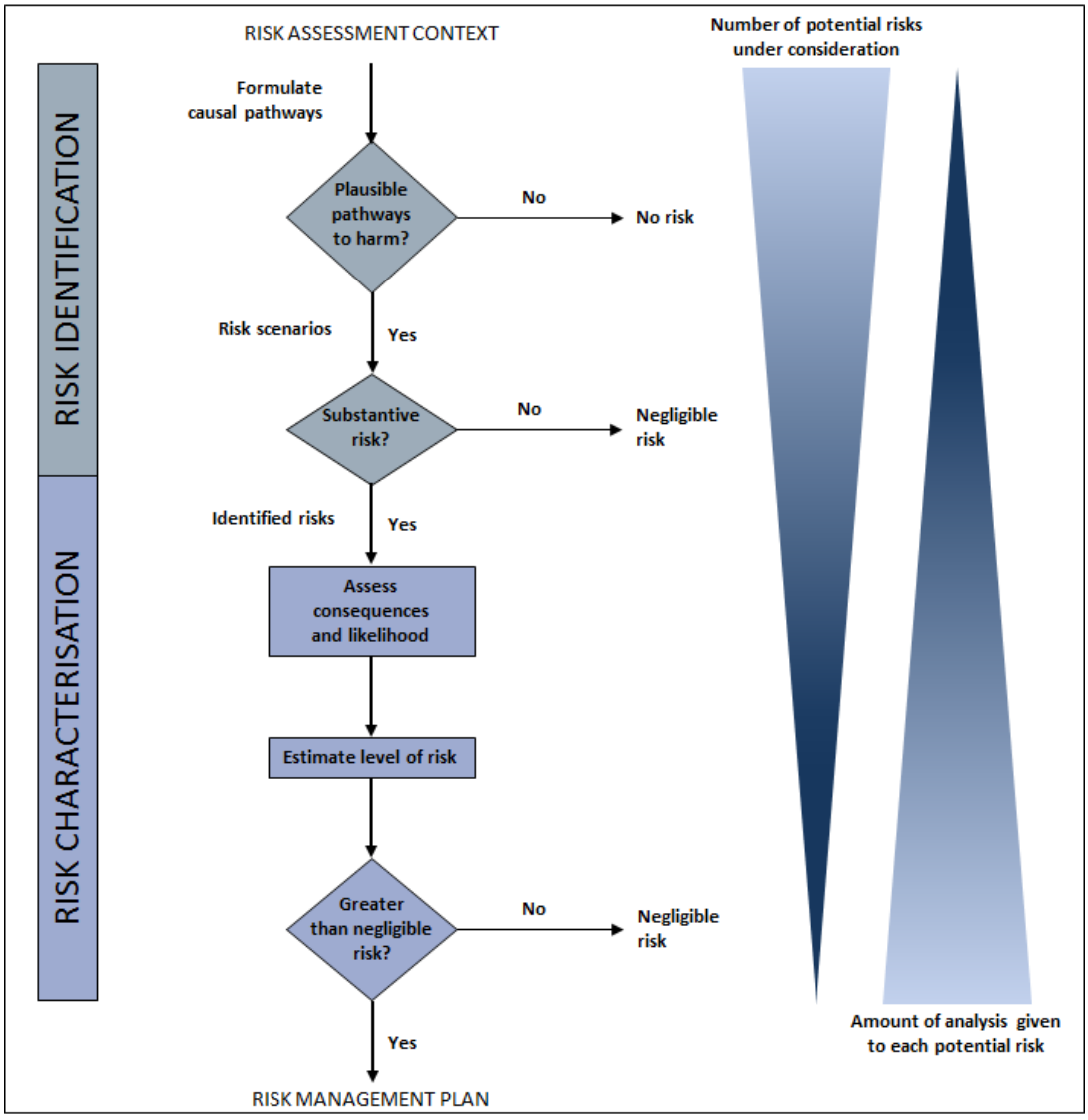

Figure 2 The risk assessment process

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

171. 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.

172. 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.

173. 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.

174. 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

175. 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.

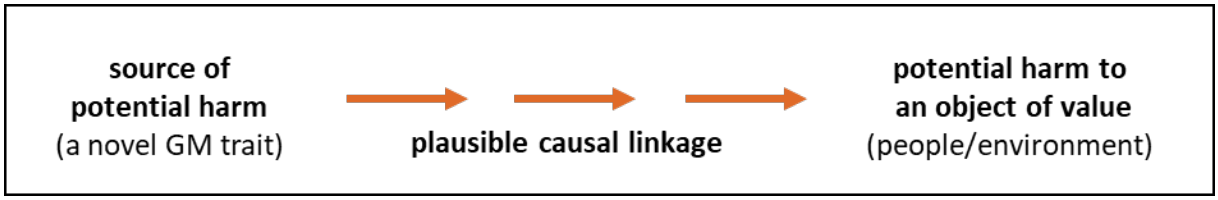

Figure 3 Components of a risk scenario

176. 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 GMOs, and
- the characteristics of the parent organism(s).

2.1 Risk source

177. 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.

178. As discussed in Chapter 1, Section 6.1, the GM canola lines proposed for release are the result of conventional breeding between MS11, RF3 and MON 88302 canola. These lines have been modified by the introduction of separate genes for tolerance to the herbicides glufosinate and glyphosate, as well as for a hybrid breeding system comprising genes for male sterility and fertility restoration. The introduced genes and their encoded proteins are considered further as potential sources of risk. The risk assessment will primarily focus on the two GM lines MS11 × RF3 and MS11 × RF3 × MON 88302 as these are expected to be cultivated on a much larger scale than the intermediate parental lines MS11

x MON 88302 and RF3 x MON 88302, although many of the considerations will apply equally to these intermediate parental lines.

179. The introduced genes are controlled by introduced regulatory sequences. These regulatory sequences are derived from common plants, soil bacteria and plant viruses (Table 5). 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 lines grown commercially in Australia and overseas, without reports of adverse effects. Hence, potential risks from the regulatory elements will not be considered further.

180. The genetic modifications have the potential to cause unintended effects in several ways including altered expression of endogenous genes by random insertion of introduced DNA in the genome, increased metabolic burden due to expression of the introduced 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

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

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

182. 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

183. The geographic range of non-GM 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 the GM canola plants more tolerant to abiotic stresses that are naturally encountered in the environment and are therefore unlikely to alter the potential distribution of the GM canola plants. Also, as discussed in Chapter 1, Section 6.2.3, the response of MS11 × RF3

and MS11 × RF3 × MON 88302 canola to abiotic factors is considered equivalent to the non-GM counterpart. Therefore, tolerance to abiotic stresses will not be assessed further.

2.2.2 Development of herbicide resistant weeds through selective pressure

184. There is some potential for development of herbicide resistant weeds if a herbicide tolerant canola and its corresponding herbicide are used inappropriately. The repetitious use of a single herbicide, or herbicide group, 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).

185. The genetic modification to the GM canola lines proposed for release confers tolerance to glufosinate and glyphosate herbicides. Four glufosinate-resistant weed species have been identified overseas, while at least 53 glyphosate-resistant weed species have been reported around the world with 21 of them also present in Australia (Chapter 1, Section 7.3.4).

186. The risk of 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 GM 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

187. 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 5.1.6.

188. If the GM herbicide tolerant canola lines are to be commercially cultivated in Australia, the potential toxicity of glufosinate and glyphosate herbicides and their 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 and glyphosate herbicides and their metabolites will not be further considered in this risk assessment.

2.2.4 Horizontal gene transfer

189. 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

190. 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

191. 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).

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

2.4 Postulated risk scenarios

193. Five risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 13 and discussed in depth in Sections 2.4.1 to 2.4.5. Postulation of risk scenarios considers impacts of the GM canola or its products on people undertaking the dealings, as well as impacts on people and the environment exposed to the GM canola or its products as the result of commercial use or the spread and persistence of plant material.

194. 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 13 Summary of risk scenarios from the proposed dealings

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced genes for herbicide tolerance and hybrid breeding system	Commercial cultivation of GM canola expressing the introduced genes ⬇ Exposure of people and other organisms via contact or consumption of GM 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 lines containing the introduced genes have a history of safe use. • The introduced genes and proteins are widespread in the environment.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
2	Introduced genes for herbicide tolerance	Commercial cultivation of GM canola lines expressing the introduced genes ⬇ Establishment of volunteer GM canola plants in agricultural areas ⬇ Reduced effectiveness of weed management measures to control volunteer GM canola plants	• Reduced establishment or yield of desirable agricultural crops, or • Increased reservoir for pests or pathogens	No	• The genetic modifications only give an advantage to the GM canola plants in managed environments, where glufosinate and/or glyphosate herbicides is applied. • The GM canola lines can be controlled using integrated weed management.
3	Introduced genes for herbicide tolerance	Commercial cultivation of GM canola lines expressing the introduced genes ⬇ Dispersal of GM 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 canola lines are similar to non-GM canola with respect to the intrinsic characteristics contributing to spread and persistence of canola. • The GM canola is susceptible to the biotic and abiotic stresses that normally restrict the geographic range and persistence of canola. • The GM canola can be controlled using integrated weed management.
4	Introduced genes for herbicide tolerance	Commercial cultivation of GM canola lines in agricultural area ⬇ Cross-pollination with other canola, including canola with other herbicide tolerance traits ⬇ Establishment of hybrid GM canola plants expressing the herbicide tolerance genes 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 GMOs and other canola 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 herbicide tolerance	Commercial cultivation of GM canola lines in agricultural area ⬇ Cross-pollination with sexually compatible species ⬇ Establishment of hybrid GM Brassica plants expressing the herbicide tolerance genes as volunteers or Introgression of the introduced herbicide tolerance genes into agricultural weeds ⬇ Establishment of hybrids expressing the herbicide tolerance genes ⬇ Reduced effectiveness of weed management measures to control hybrids expressing the herbicide tolerance genes	• Reduced establishment or yield of desirable agricultural crops	No	• Hybrids between the GM canola and Brassica crop or weed species would occur at 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 herbicide tolerance and hybrid breeding system
Causal pathway	⬇ Commercial cultivation of GM canola lines expressing the introduced genes ⬇ Exposure of people and other organisms via contact or consumption of GM canola plants or products ⬇
Potential harm	Increased toxicity or allergenicity for people OR Increased toxicity for other desirable organisms

Risk source

195. The source of potential harm for this postulated risk scenario is the introduced genes for herbicide tolerance and hybrid breeding system.

Causal pathway

196. The applicant proposes that the GM canola lines would be cultivated on a commercial scale in all Australian canola growing areas. The herbicide tolerance genes *cp4 epsps* and *bar* are expressed in all parts of the GM canola plant at all developmental stages including leaf, stem, root and seed. Expression of the *barnase* gene is restricted to the anthers and the *barstar* gene is mainly expressed in the anthers but also in other tissues at low levels (Chapter 1, Section 6.2.2).

197. The GM canola lines would enter general commerce and be used in the same ways as non-GM canola. The general public could be exposed to oil from the GM canola, which would be sold for human consumption. However, refined canola oil is unlikely to contain any protein (FSANZ, 2017).

198. People could be exposed to wind-borne GM 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), so this route of exposure would mainly apply to people who enter or pass close to GM canola fields during flowering.

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

200. Livestock could be exposed when consuming the GM canola lines as forage, whole seed or seed meal.

201. Wild animals and birds could enter canola fields and feed on GM canola seed or other plant parts. Pollinators such as bees would be exposed to nectar and pollen from the GM canola lines. 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 canola lines and plant material derived from them.

Potential harm

202. 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.

203. The *cp4 epsps*, *bar*, *barnase* and *barstar* genes introduced into the GM canola lines 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 5.1.5), and do not change the biochemical composition of the GM canola seeds (Chapter 1, Section 6.2.4).

204. FSANZ has determined that food derived from the parental GM canola lines, MS11, RF3 and MON 88302, is as safe for human consumption as food derived from conventional (non-GM) canola varieties (Chapter 1, Section 5.2). These approvals also cover MS11 × RF3 canola, MS11 × RF3 × MON 88302 canola and the intermediate parental lines MS11 x MON 88302 and RF3 x MON 88302. The parental GM lines have also been approved for food and/or feed use in other countries, including Canada, the Philippines, South Korea and the USA (Chapter 1, Section 3.3). Compositional analysis of seed from MS11 × RF3 and MS11 × RF3 × MON 88302 canola also confirmed that seed from these GM canola lines are compositionally equivalent to seed from conventional canola varieties (Chapter 1, Section 6.2.4).

205. There have been no reported adverse effects on human or animal health from these GM canola lines (Chapter 1, Section 3.1.2) or other commercial GM crops with the same introduced herbicide tolerance genes (Chapter 1, Section 5.1.5).

206. The introduced genes were isolated from common soil bacteria (Chapter 1, Section 7.4). Homologous EPSPS proteins that perform the identical biochemical reaction to the introduced CP4 EPSPS protein occur in all plants and many other microorganisms (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

207. Risk scenario 1 is not identified as a substantive risk because the introduced 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, and the introduced genes and proteins are widespread in

the environment. 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 herbicide tolerance
Causal pathway	⬇ Commercial cultivation of GM canola lines expressing the introduced genes ⬇ Establishment of volunteer GM canola plants in agricultural areas ⬇ Reduced effectiveness of weed management measures to control volunteer GM canola plants ⬇
Potential harm	Reduced establishment or yield of desirable agricultural crops OR Increased reservoir for pests or pathogens

Risk source

208. The source of potential harm for this postulated risk scenario is the introduced genes for herbicide tolerance.

Causal pathway

209. The applicant proposes that the GM canola lines 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). Studies in the USA and Canada indicated no meaningful differences between MS11 × RF3 or MS11 × RF3 × MON 88302 and non-GM canola with respect to the intrinsic characteristics contributing to spread and persistence (eg seed production, pod shattering and competitiveness; Chapter 1, Section 6.2.3); it would be expected to produce similar numbers of volunteers as other canola. This expectation is also consistent with the finding of low levels of GM Roundup Ready® volunteer canola along road sides in the Esperance region of WA after one year of commercial production (see Chapter 1, Section 5.3).

210. Volunteer canola plants are likely to occur following dispersal of GM canola seeds within agricultural areas (Chapter 1, Section 4.2). Short-range dispersal of canola seed into field margins or adjacent fields could occur via pod shattering or transport of canola plant material from windrows by strong winds (OGTR, 2017). Short to medium-range dispersal of canola seed within agricultural areas could be mediated by human activities such as movement of agricultural machinery used during canola sowing or harvest. Dispersal of viable canola seed by animals or birds via consumption and excretion is also possible at very low levels (OGTR, 2017).

211. MS11 × RF3 canola only has a survival advantage in the presence of glufosinate, while MS11 × RF3 × MON 88302 only has a survival advantage in the presence of glufosinate or glyphosate or both herbicides. Glyphosate is widely used for weed control in broad-acre agriculture, horticulture and other weed management situations, whereas glufosinate is not widely used in broad-acre cropping or management along roadsides. Neither herbicide would be effective in controlling canola volunteers in situations where MS11 × RF3 × MON 88302 canola had been grown previously. The presence of MS11 × RF3 × MON 88302 canola volunteers in agricultural areas has implications for the choice of herbicide(s) in situations where glyphosate is the principal weed control strategy. Crop Management Plans have been developed separately by BASF and Bayer CropScience for MS11 × RF3 and MS11 × RF3 × MON 88302 canola, respectively (see also Section 7.3.4). These CMPs are to be followed by canola growers when growing the GM canola lines. The CMPs address issues such as minimising and

managing canola volunteers in crops following GM herbicide tolerant canola in a rotation, and minimising the development of herbicide resistant weeds.

212. All herbicides sold in Australia must be labelled with their mode of action for the purpose of resistance management (APVMA website, accessed November 2020). The mode of action is indicated by a letter code on the product label. Glufosinate is a group N herbicide and is registered for the control of canola volunteers in Australia, along with herbicides belonging to eight other mode of action groups (Chapter 1, Section 4.2.4). Glyphosate is a mode of action Group M herbicide. Herbicides from different mode of action groups or products with multiple mode of action groups could be used to control MS11 × RF3 and MS11 × RF3 × MON 88302 canola volunteers. Specifically, herbicides from groups B, C, F, G, H, I, L and Q are available to control volunteer canola in various crop and non-crop situations (Australian Oilseeds Federation, 2019). In addition, combinations of herbicides from multiple mode of action groups (B+G, B+I, C+F, C+H, C+I, F+I, G+I, H+I, L+Q and C+F+I) are registered for use on canola volunteers. Further details of registered herbicide products are available on the APVMA PubCRIS database.

213. MS11 × RF3 canola volunteers only have a survival advantage over non-GM canola volunteers in the presence of glufosinate herbicides, while MS11 × RF3 × MON 88302 canola only have the advantage in the presence of glufosinate and glyphosate herbicides. They are as susceptible as non-GM canola to all herbicides other than glufosinate and glyphosate herbicides. The GM 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

214. Volunteer canola (non-GM and GM) is a weed of agricultural production systems (Groves et al., 2003). If left uncontrolled, volunteer canola plants could establish and compete with other crops. However, as discussed above, there are alternative methods to control the GM volunteers and, therefore, the number of volunteers persisting in agricultural areas is likely to be low, further minimising the likelihood of reduced establishment or yield of crops. GM canola volunteers that are effectively controlled would not be expected to cause greater harm to desired crops than non-GM canola volunteers that are effectively controlled.

215. Canola crops are susceptible to a range of pests and diseases (Chapter 1, Section 7.3.3). Volunteer canola can act as a reservoir for canola pests and pathogens. For example, volunteer canola plants can be a source of diamondback moth infestation and can act as a reservoir for viral and fungal pathogens of canola (GRDC, 2009). Characterisation of the GM canola lines did not reveal any significant differences between the GM canola lines and conventional canola for disease stress or insect stress ratings (Chapter 1, Section 6.2.3). Effective control of canola volunteers (both GM and non-GM) reduces the potential for volunteers to act as reservoirs for pests and diseases.

Conclusion

216. Risk scenario 2 is not identified as a substantive risk because the genetic modification only gives an advantage to the GM canola plants in managed environments where glufosinate and/or glyphosate herbicides is applied, and because the GM canola lines 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
Causal pathway	⬇ Commercial cultivation of GM canola lines expressing the introduced genes ⬇ Dispersal of GM 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

217. The source of potential harm for this postulated risk scenario is the introduced genes for herbicide tolerance.

Causal pathway

218. The applicant proposes to grow the GM canola lines on a commercial scale. After harvest, the GM 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 or storage sites.

219. If transport routes passed through or were near nature reserves, dispersal of canola seeds into nature reserves could occur via spillages, or GM canola could spread into nature reserves after establishing along transport routes (Bailleul et al., 2012). However, as discussed in Chapter 1, Section 4.2.3, surveys of roadside canola typically only found feral canola plants within five metres of the edge of the road. Feral canola plants are often observed growing on roadsides or railway easements in Australia. These canola populations are thought to be reliant on re-supply of seed from spillages, rather than forming self-sustaining weed populations.

220. Whole seeds could be used as livestock feed and feral GM canola could potentially establish in and around animal feeding areas, which are also included in intensive use areas.

221. Canola seed is small and thus easily dispersed. Canola fruits can shatter some seeds prior to and during harvest, allowing for the establishment of volunteers in areas near the cultivated field (Ellstrand, 2018). Dispersal of viable canola seed into nature reserves by animals or 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).

222. If seed from the GM canola lines is dispersed into intensive use areas or nature reserves, the seeds could germinate and establish a population of GM plants. A study on *Arabidopsis* showed that overexpressing an *epsps* gene could significantly enhance fecundity of the GM plants without glyphosate application (Fang et al., 2018), which could contribute to greater weediness. However, GM canola overexpressing the *epsps* gene has been grown in the Australian environment since 2002 and there have been no reports of increased weediness relative to non-GM canola. Consistent with this, the GM canola lines proposed for release are similar to non-GM 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 canola. The genetic

modifications are also not expected to alter the tolerance of GM plants to biotic or abiotic stresses that normally restrict the geographic range and persistence of canola (Chapter 1, Sections 7.2 and 7.3). Therefore, feral GM canola would rarely have a survival advantage over non-GM canola and is not expected to be more persistent than non-GM canola.

223. The traits of glufosinate and glyphosate tolerance could affect a GM plant's tolerance to weed management practices in areas where either or both of these herbicides are used. The main herbicide used for roadside weed management in Australia is glyphosate (Storrie, 2018). Glyphosate would not be effective in controlling feral MS11 × RF3 × MON 88302 canola populations due to the expression of the introduced *cp4 epsps* gene. Broad application of glyphosate in intensive use areas could potentially promote the establishment of feral GM canola due to reduction of competition. An Australian study found that under favourable climatic conditions, and in circumstances where other roadside weeds are controlled by glyphosate, roadside populations of glyphosate tolerant GM canola could persist for at least three years (Busi and Powles, 2016).

224. In this context it should be noted that there are already glyphosate resistant weedy species such as annual ryegrass, barnyard grass, brome grass, fleabane and windmill grass present on Australian roadsides and railway lines. The Australian Glyphosate Sustainability Working Group recommends a number of tactics to deal with glyphosate resistant weeds in non-agricultural areas, including strategic use of alternative herbicide modes of action, physical control practices aimed at weed seed set prevention, and planting or managing other species to compete with weeds (AGSWG, 2012). These strategies would also be effective in controlling feral GM canola.

225. In nature reserves where glufosinate or glyphosate are not used for weed control, the GM canola lines would not be expected to have any survival advantage over non-GM canola. The study by Busi and Powles (2016) also found that when glyphosate tolerant GM canola seeds were dispersed into two natural areas, feral canola populations persisted for 0 and 3 years, respectively, prior to extinction. This is consistent with the fact that canola is not a persistent weed in natural undisturbed habitats in Australia (Chapter 1, Section 4.2.3).

Potential harm

226. If the GM canola lines expressing the introduced genes for herbicide tolerance 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 canola could also potentially reduce services from the land use by decreasing the amenity of nature reserves for nature-based tourism.

227. Canola can grow to a height of 1.5 m along roadsides (OGTR, 2017) and is highly visible when in flower. Feral canola on roadsides or along railway lines could reduce services from the land use by obstructing lines of sight around corners or to signs. The Western Australian Department of Parks and Wildlife lists feral canola as one of 60 weeds that threaten rail and roadside vegetation by lowering the biodiversity and aesthetic value of the verge, and recommends that management of these weeds be a priority along roads of high conservation value (Roadside Conservation Committee, 2014). However, a latest national weeds data collection survey conducted by ABARES showed that canola is not listed as an established weed causing agricultural, social or environmental impacts by weed managers around Australia (Ng et al., 2021), indicating that feral canola was not a weed of nationwide concern.

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

Conclusion

229. Risk scenario 3 is not identified as a substantive risk because the GM canola lines are similar to non-GM canola with respect to the intrinsic characteristics contributing to spread and persistence of canola, are susceptible to the biotic or abiotic stresses that normally restrict the geographic range and

persistence of canola, and can be controlled using integrated weed management. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.4 Risk scenario 4

Risk source	Introduced genes for herbicide tolerance
Causal pathway	⬇ Commercial cultivation of GM canola lines in agricultural areas ⬇ Cross-pollination with other canola, including canola with other herbicide tolerance traits ⬇ Establishment of hybrid GM canola plants expressing the herbicide tolerance 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

230. The source of potential harm for this postulated risk scenario is the introduced genes for herbicide tolerance.

Causal pathway

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

232. Outcrossing rates between neighbouring commercial canola fields in Australia are less than 0.1% averaged over whole fields (Rieger et al., 2002). Correspondingly low levels of hybridisation are expected between the GM canola lines and other canola.

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

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

235. Crossing may also occur between the GM lines and non-GM herbicide tolerant varieties. As discussed in Chapter 1, Section 7.3.1, there are currently three herbicide tolerance traits in Australian canola varieties:

- non-GM triazine tolerance (TT)
- non-GM imidazolinone tolerance (IMI; Clearfield®)
- GM glyphosate tolerance (GT; Roundup Ready®, TruFlex®).

236. 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). If the GM canola lines proposed for release were to cross with the TT, IMI and GT canola, this could result in a canola with tolerance to four herbicides. This has been a possibility since the approval of InVigor® canola and Roundup Ready® canola in 2003, so approval of the GM canola lines for commercial release would not add a new trait in terms of combinations of herbicide tolerance in canola 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 canola lines proposed for release were widely grown, the likelihood of multiple-herbicide tolerant hybrids as volunteers could increase.

Potential harm

237. If left uncontrolled in agricultural areas, volunteer GM canola plants could establish and compete with other crops. Hybrid canola volunteers with multiple herbicide tolerance traits may not be effectively controlled by existing weed management measures, particularly where herbicide tolerance traits acquired by pollen flow were not anticipated. In addition, surviving volunteer canola could act as a reservoir for canola pests or pathogens, as described in Risk scenario 2. As a result, the establishment and yield of desirable agricultural crops might be reduced.

238. However, additional herbicide tolerance traits are not expected to provide a survival advantage to the GM canola, except in the presence of the herbicides to which they are tolerant. Canola volunteers that have 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 or their non-GM counterparts (Beckie et al., 2004).

239. In addition, the applicant will have CMPs ready for canola growers to follow when growing the GM canola lines (Chapter 1, Sections 7.1 and 7.3.4). These include management strategies that aim to control canola volunteers, minimise gene flow, and prevent or delay the development of herbicide resistant weeds.

240. In summary, if management practices are used effectively, hybrid canola volunteers are expected to be present at very low densities and no greater numbers than for non-GM canola. Small numbers of volunteers would have limited capacity to cause adverse effects.

Conclusion

241. Risk scenario 4 is not identified as a substantive risk because hybrids between the GM canola lines and other canola would be generated 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.

2.4.5 Risk scenario 5

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

Risk source

242. The source of potential harm for this postulated risk scenario is the introduced genes for herbicide tolerance.

Causal pathway

243. The applicant proposes that the GM canola be cultivated on a commercial scale in all canola growing areas. This could bring it into proximity to other Brassica crop species, such as vegetables, forage crops and Indian mustard, as well as related weed species.

Interactions with Brassica crop species

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

245. Hybrids between *B. napus* and *B. juncea* have been observed in the field, are fertile, and often have high fitness (Liu et al., 2010). Cross-pollination between *B. napus* and *B. rapa* occurs frequently in the field if plants of the two species are in proximity, and the hybrids are vigorous and fertile, although with reduced pollen viability (Warwick et al., 2003). A report also showed that an herbicide tolerance trait from a commercial canola crop was transferred to, and stably maintained in, a wild *B. rapa* population for at least six years (Warwick et al., 2008). Hybrids between *B. napus* and *B. oleracea* have been detected at low levels in wild populations (Ford et al., 2006).

246. Based on the data above, hybridisation between GM canola and other Brassica crop species is expected to occur if the GM canola lines are released. However, the frequency of inter-species crossing would be lower than the frequency of crossing between the GM canola and other canola plants, both because there is greater sexual compatibility between *B. napus* plants than between *B. napus* and other species, and because canola is far more widely grown than other Brassica crops (ABARES, 2015). In Risk Scenario 4, it was considered that hybridisation between the GM canola lines and other canola would occur at low levels, so hybridisation between the GM canola lines and other Brassica crop species is likely to occur at very low levels.

247. Volunteer plants that are hybrids between the GM canola lines and other Brassica crop species would not be controlled by the application of glufosinate and/or glyphosate 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 herbicide tolerance genes is not expected to alter intrinsic characteristics contributing to spread and persistence, or to alter the tolerance of GM plants to biotic or abiotic stresses. Therefore, GM hybrid volunteers would not be expected to be more invasive or persistent than hybrids between non-GM canola and other Brassica crop species.

Interactions with Brassicaceae weeds

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

249. Naturally occurring hybrids between *B. napus* and weed species (wild radish, *Raphanus raphanistrum*; Buchan weed, *Hirschfeldia incana*; and charlock, *Sinapis arvensis*) have been observed at very low levels (Chapter 1, Section 7.3.1). Thus, introgression of the herbicide genes from the GM canola lines into wild radish or Buchan weed populations is highly unlikely.

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

251. In the highly unlikely event that herbicide tolerance genes were 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. The GM weeds would not be controlled by the application of glufosinate and/or glyphosate 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

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

253. Hybrid GM volunteers and weeds 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

254. 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 traits were introgressed into a population of one of these weeds, it would increase the difficulty of weed management, particularly where herbicide tolerance traits were not anticipated. These GM weeds could impact the agricultural environment by reducing the establishment or yield of desired crops.

255. It should be noted that weeds can evolve herbicide resistance through natural mechanisms due to selective pressure. There are reports of wild radish populations in Australia that have acquired resistance to one or more of five classes of herbicides, including glyphosate (Ashworth et al., 2014; Heap, 2020). If wild radish did acquire a herbicide tolerance gene from GM canola, it would be no more difficult to control than wild radish that had naturally evolved herbicide resistance.

Conclusion

256. Risk scenario 5 is not identified as a substantive risk because hybrids between the GM canola lines and Brassica crop or weed species would occur at very low levels, hybrids can be controlled using integrated weed management, and it is highly unlikely that a GM herbicide tolerance gene would introgress into Brassicaceae weed species. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

257. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis[7]. 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.

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

259. MS11 × RF3 canola has been approved by the Regulator for limited and controlled release (field trial) under licence DIR 104. The RARMP for DIR 104 identified additional information that may be required for a large scale or commercial release of MS11 × RF3 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 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.

260. Uncertainty can arise from a lack of experience with the GMOs. MS11 x RF3 proposed for release has only been grown in Australia under a limited and controlled (field trial) licence, and MS11 × RF3 × MON 88302 has not yet been approved by the Regulator for any intentional release in

[7] 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.

Australia. As the GM canola lines would be grown in diverse environmental and agronomic conditions across different agro-ecological zones in Australia, their behaviour in terms of abiotic and biotic stress tolerance in various canola planting areas remains to be observed. However, the level of uncertainty is considered to be low, given that the GM canola lines and earlier generation GM canola containing the *bar, cp4 epsps, barnase* and *barstar* genes have been widely grown as commercial crops in the USA and Canada for many years without adverse effects on human health and safety or the environment.

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

262. Post release review (PRR) will be used to address uncertainty regarding future changes to knowledge about the GMOs 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

263. 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.

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

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

265. 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 13.

266. 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.

Chapter 3 Risk management plan

Section 1 Background

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

268. 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.

269. 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.

270. 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

271. 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 canola lines. 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 containment measures are required to treat these negligible risks.

Section 3 General risk management

272. 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

273. 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.

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

275. 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

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

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

277. Any person, including the licence holder, could conduct any permitted dealing with the GMO.

3.4 Reporting requirements

278. The licence obliges 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.

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

280. 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

281. 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.

282. 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

283. 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.

284. The Regulator engages in ongoing oversight of licences to take account of future findings or changes in circumstances. This ongoing oversight will be achieved through post release review (PRR) activities. The three components of PRR are:

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

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

285. 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 assessment of future applications involving similar GMOs.

4.2 Requirement to monitor specific indicators of harm

286. 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.

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

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

289. 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 178. However, specific indicators of harm may also be identified during later stages, e.g. through either of the other components of PRR.

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

4.3 Review of the RARMP

291. 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 RARMP

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

293. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, general conditions have been imposed to ensure that there is ongoing oversight of the release.

References

ABARES (2015). Australian Crop Report No. 174, June 2015. (Canberra: Australian Bureau of Agricultural and Resource Economics and Sciences).

ABARES (2020). Australian crop report: June 2020. (Canberra, Australia: Australian Bureau of Agricultural and Resource Economics and Sciences).

Adamczyk-Chauvat, K., Delaunay, S., Vannier, A., François, C., Thomas, G., Eber, F., Lodé, M.*, et al.* (2017). Gene introgression in weeds depends on initial gene location in the crop: *Brassica napus*–*Raphanus raphanistrum* model. Genetics *206*, 1361-1372.

Advanta Seeds (2019). Hyola XC stewardship guide. (Australia: Advanta Seeds Pty Ltd).

Agrisearch (2001). A physical survey of representative Australian roadside vegetation to evaluate the incidence and distribution of canola and key Brassicaceae weeds. Report No. 0118/1, Monsanto Company, Saint Louis, Missouri, USA.

AGSWG (2012). Sustainable glyphosate use in winter grain cropping. (The Australian Glyphosate Sustainability Working Group).

Anon. (2016). Detailed insert characterization and confirmation of the absence of vector backbone sequence in *Brassica napus* MS11. Document number M-547543-01. (Bayer, unpublished).

ANZFA (2000). Final risk analysis report - Application A363: Food produced from glyphosate-tolerant canola line GT73. (Canberra, Australia: Australia New Zealand Food Authority).

ANZFA (2001a). Final assessment report - Application A372: Oil derived from glufosinate-ammonium tolerant canola lines Topas 19/2 and T45 and oil derived from glufosinate-ammonium tolerant and pollination controlled lines MS1, MS8, RF2 and RF3. (Canberra, Australia: Australia New Zealand Food Authority).

ANZFA (2001b). Final risk analysis report - Application A372: Oil derived from glufosinate-ammonium tolerant canola lines Topas 19/2 and T45 and oil derived from glufosinate-ammonium tolerant and pollination controlled lines MS1, MS8, RF2 and RF3. (Canberra, Australia: Australia New Zealand Food Authority).

Asaduzzaman, M., Pratley, J.E., Luckett, D., Lemerle, D., and Wu, H. (2020). Weed management in canola (*Brassica napus* L): a review of current constraints and future strategies for Australia. Archives of Agronomy and Soil Science *66*, 427-444.

Ashworth, M.B., Walsh, M.J., Flower, K.C., and Powles, S.B. (2014). Identification of the first glyphosate-resistant wild radish (*Raphanus raphanistrum* L.) populations. Pest Manag Sci *70*, 1432-1436.

Australian Oilseeds Federation (2019). Canola volunteer control 2019.

Bailleul, D., Ollier, S., Huet, S., Gardarin, A., and Lecomte, J. (2012). Seed spillage from grain trailers on road verges during oilseed rape harvest: an experimental survey. PLoS One *7*, e32752.

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

Baldacci-Cresp, F., Houbaert, A., Dabire, A.M., Mol, A., Monteyne, D., El Jaziri, M., Van Melderen, L., *et al.* (2016). *Escherichia coli mazEF* toxin-antitoxin system as a tool to target cell ablation in plants. Journal of Molecular Microbiology Biotechnology *26*, 277-283.

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

BASF (2018a). MS11 × RF3 × MON 88302 *Brassica napus* – Expression of Barnase, Barstar, PAT/bar and CP4 EPSPS proteins in field samples grown in Canada and USA during 2017. Study ID: 17-RSATS007. (BASF Agricultural Solutions, unpublished).

BASF (2018b). Structual stability analysis of MS11 × RF3 × MON 88302 *Brassica napus*. Study ID: 18-RSATS003. (BASF Agricultural Solutions, unpublished).

BASF (2019a). MS11 × RF3 × MON 88302 *B. napus* – Composition assessment of field samples grown in Canada and the USA during 2017. Report ID: 17-RSATS014-C. (BASF Agricultural Solutions, unpublished).

BASF (2019b). MS11 × RF3 × MON 88302 *Brassica napus* – Agronomic assessment of *Brassica napus* grown in Canada and the USA during 2017. Report ID: 17-RSATS014-A. (BASF Agricultural Solutions, unpublished).

Bayer (2015). MS11 × RF3, MS11, and RF3 *Brassica napus* – Protein expression analyses of field samples grown in Canada and the USA during 2014. Study Report No: 14-RSBBS028. (Bayer CropScience, unpublished).

Bayer (2016a). MS11 × RF3 *B. napus* – Agronomic assessment of MS11 × RF3 *B. napus* grown in Canada and the USA during 2014. Study No: 14-RSBBS019. (Bayer CropScience, unpublished).

Bayer (2016b). MS11 × RF3 *B. napus* – Composition analysis of field samples grown in Canada and the USA during 2014. Report ID: 17-RSATS014-C. (Bayer CropScience, unpublished).

Bayer (2016c). Structural stability analysis of MS11 × RF3 *Brassica napus*. Study Report No: 15-RSBBS025. (Bayer CropScience, unpublished).

Beckie, H.J., and Owen, M.D.K. (2007). Herbicide-tolerant crops as weeds in North America. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 2 (044). (CABI Publishing 2007. Available online).

Beckie, H.J., Séguin-Swartz, G., Warwick, S.I., and Johnson, E. (2004). Multiple herbicide–resistant canola can be controlled by alternative herbicides. Weed Science *52*, 152-157.

Beckie, H.J., and Warwick, S.I. (2010). Persistence of an oilseed rape transgene in the environment. Crop Protection *29*, 509-512.

Beckie, H.J., Warwick, S.I., Nair, H., and Séguin-Swartz, G. (2003). Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). Ecological Applications *13*, 1276-1294.

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

Bentley, R. (1990). The shikimate pathway - a metabolic tree with many branches. Critical Reviews in Biochemistry and Molecular Biology *25*, 307-384.

Benz, J., and Meinhart, A. (2014). Antibacterial effector/immunity systems: it's just the tip of the iceberg. Current Opinion in Microbiology *17*, 1-10.

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

Busi, R., Vila-Aiub, M.M., Beckie, H.J., Gaines, T.A., Goggin, D.E., Kaundun, S.S., Lacoste, M.*, et al.* (2013). Herbicide-resistant weeds: from research and knowledge to future needs. Evolutionary Applications *6*, 1218-1221.

CANBR (2019). Census of the Flora of the Australian Capital Territory, version 4.1. (Centre for Australian National Biodiversity Research).

CFIA (1996). DD1996-17: Determination of environmental safety of Plant Genetic Systems Inc.'s (PGS) novel hybridization system for rapeseed (*Brassica napus* L.). (Canadian Food Inspection Agency).

CFIA (2012). Decision Document 2012-90: Determination of the safety of Monsanto Canada Inc. canola (*Brassica napus*) event MON 88302. (Canadian Food Inspection Agency).

CFIA (2018). Decision Document DD2018-121: Determination of the safety of Bayer CropScience Inc.'s canola (*Brassica napus* L.) event MS11. (Canadian Food Inspection Agency).

Chalhoub, B., Denoeud, F., Liu, S., Parkin, I.A., Tang, H., Wang, X., Chiquet, J.*, et al.* (2014). Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. Science *345*, 950-953.

Chevre, A.M., Eber, F., Jenczewski, E., Darmency, H., and Renard, M. (2003). Gene flow from oilseed rape to weedy species. Acta Agriculturae Scandinavica, Section B, Soil and Plant Science *53*, 22-25.

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

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

Clayton, G.W., Harker, K.N., Johnston, A.M., and Turkington, K.T. (1999). Response of hybrid canola to seeding rate, fertility and time of weed removal. Paper presented at: The Regional Institute Ltd).

Colton, B., and Potter, T.D. (1999). History. In Canola in Australia: the first 30 years, P.A. Salisbury, T.D. Potter, G. McDonald, and A.G. Green, eds. (Organising Committee of the 10th International Rapeseed Congress).

Crawley, M.J., and Brown, S.L. (2004). Spatially structured population dynamics in feral oilseed rape. Proceedings of the Royal Society of London Series B: Biological Sciences *271*, 1909-1916.

Crawley, M.J., Hails, R.S., Rees, M., Kohn, D.D., and Buxton, J. (1993). Ecology of transgenic oilseed rape in natural habitats. Nature *363*, 620-623.

DAFWA (2010). 2010 GM canola audit program. (Report produced by Department of Agriculture and Food Western Australia. Available online).

Darmency, H., and Fleury, A. (2000). Mating system in *Hirschfeldia incana* and hybridisation to oilseed rape. Weed Research *40*, 231-238.

Darmency, H., Lefol, E., and Fleury, A. (1998). Spontaneous hybridisations between oilseed rape and wild radish. Molecular Ecology *7*, 1467-1473.

De Block, M., and Debrouwer, D. (1993). Engineered fertility control in transgenic *Brassica napus* L.: Histochemical analysis of anther development. Planta *189*, 218-225.

de Salas, M.F., and Baker, M.L. (2018). A Census of the Vascular Plants of Tasmania, including Macquarie Island. (Hobart, Australia: Tasmanian Herbarium, Tasmanian Museum and Art Gallery).

Depicker, A., Stachel, S., Dhaese, P., Zambryski, P., and Goodman, H.M. (1982). Nopaline synthase: transcript mapping and DNA sequence. Journal of Molecular and Applied Genetics *1*, 561-573.

Dhaese, P., De Greve, H., Gielen, J., Seurinck, L., Van Montagu, M., and Schell, J. (1983). Identification of sequences involved in the polyadenylation of higher plant nuclear transcripts using Agrobacterium T-DNA genes as models. EMBO Journal *2*, 419-426.

Dignam, M. (2001). Bush, parks, road and rail weed management survey. Report No. CMD.274. (Monsanto Australia Ltd, Melbourne, Australia).

Dill, G.M. (2005). Glyphosate-resistant crops: history, status and future. Pest Management Science *61*, 219-224.

Dröge, W., Broer, I., and Pühler, A. (1992). Transgenic plants containing the phosphinothricin-*N*-acetyltransferase gene metabolize the herbicide L-phosphinothricin (glufosinate) differently from untransformed plants. Planta *187*, 142-151.

Edginton, M. (2019). Brassicaceae. In Census of the Queensland Flora 2019, G.K. Brown, and P.D. Bostock, eds. (Queensland Department of Environment and Science, Queensland Government).

Ellstrand, N.C. (2018). "Born to Run"? Not Necessarily: Species and Trait Bias in Persistent Free-Living Transgenic Plants. Front Bioeng Biotechnol *6*, 88.

Environment Canada, and Health Canada (2015). Final screening assessment for DSL *Bacillus licheniformis/subtilis* group.

Eschenburg, S., Healy, M.L., Priestman, M.A., Lushington, G.H., and Schonbrunn, E. (2002). How the mutation glycine96 to alanine confers glyphosate insensitivity to 5-enolpyruvyl shikimate-3-phosphate synthase from *Escherichia coli*. Planta *216*, 129-135.

Evstigneeva, Z.G., Solov'eva, N.A., and Sidel'nikova, L.I. (2003). Methionine sulfoximine and phosphinothricin: A review of their herbicidal activity and effects on glutamine synthetase. Applied Biochemistry and Microbiology *39*, 539-543.

Fang, J., Nan, P., Gu, Z., Ge, X., Feng, Y.Q., and Lu, B.R. (2018). Overexpressing exogenous 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) genes increases fecundity and auxin content of transgenic Arabidopsis plants. Front Plant Sci *9*, 233.

FAO (2014). Pesticide residues in food 2013: Joint FAO/WHO meeting on pesticide residues. (Rome, Italy: World Health Organization; Food and Agriculture Organization of the United Nations).

Fischer, G., Nachtergaele, F.O., van Velthuizen, H.T., Chiozza, F., Franceschini, G., Henry, M., Muchoney, D.*, et al.* (2021). Global agro-ecological zones (GAEZ v4) model documentation. (FAO and IIASA).

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

Franz, J.E., Mao, M.K., and Sikorski, J.A. (1997). Glyphosate, a Unique Global Herbicide, Vol American chemical society Monograph 189 (Washington, USA: American Chemical Society).

FSANZ (2005a). Final assessment report- Application A533: Food derived from glufosinate ammonium-tolerant cotton line LL25. (Canberra, Australia: Food Standards Australia New Zealand).

FSANZ (2005b). Final assessment report - Application A525: Food derived from herbicide-tolerant sugar beet H7-1. (Food Standards Australia New Zealand, available online at http://www.foodstandards.gov.au/_srcfiles/A525%20GM%20Sugar%20beet%20FAR.pdf).

FSANZ (2005c). Final assessment report - Application A543: Food derived from Insect-protected, glufosinate ammonium-tolerant corn line 59122-7. (Canberra, Australia: Food Standards Australia New Zealand).

FSANZ (2005d). Final assessment report - Application A553: Food derived from glyphosate-tolerant cotton line MON 88913. (Canberra: Food Standards Australia New Zealand).

FSANZ (2006a). Final assessment report - Application A548: Food from corn rootworm-protected & glyphosate tolerant corn MON 88017. (Food Standards Australia New Zealand, available online at http://cera-gmc.org/docs/decdocs/06-221-004.pdf).

FSANZ (2006b). Final Assessment Report, Application A575, Food Derived from Glyphosate-Tolerant Lucerne J101 and J163, 13 December 2006. Report No. A575. (Food Standards Australia New Zealand).

FSANZ (2007). Final Assessment Report - Application A592, Food Derived from Glyphosate-Tolerant Soybean MON 89788. (Food Standards Australia New Zealand, available online at http://www.foodstandards.gov.au/_srcfiles/A592_FAR_GM_Soybean_MON89788_FINAL.pdf).

FSANZ (2008). Final assessment report - Application A589: Food derived from glufosinate ammonium tolerant rice line LLRICE62. (Canberra, Australia: Food Standards Australia New Zealand).

FSANZ (2010a). Application A1028: Food derived from insect-protected & herbicide-tolerant cotton line T304-40 - Approval report. (Canberra, Australia: Food Standards Australia New Zealand).

FSANZ (2010b). Application A1040: Food derived from insect-protected and herbicide-tolerant cotton line GHB119 - Approval report. (Canberra, Australia: Food Standards Australia New Zealand).

FSANZ (2013a). Approval report - Application A1080. Food derived from herbicide-tolerant cotton line MON 88701. (Canberra, Australia: Food Standards Australia New Zealand).

FSANZ (2013b). Safety assessment - Application A1071 (Approval): Food derived from herbicide-tolerant canola line MON88302. (Food Standard Australia New Zealand.).

FSANZ (2017). A1140 – Food derived from Herbicide-tolerant Canola Line MS11: Supporting document 1 - Safety Assessment (at Approval). (Canberra, Australia: Food Standards Australia New Zealand).

Garcia-Alonso, M., Hendley, P., Bigler, F., Mayeregger, E., Parker, R., Rubinstein, C., Satorre, E.*, et al.* (2014). Transportability of confined field trial data for environmental risk assessment of genetically engineered plants: a conceptual framework. Transgenic Res *23*, 1025-1041.

Gasser, C.S., Winter, J.A., Hironaka, C.M., and Shah, D.M. (1988). Structure, expression, and evolution of the 5-enolpyruvylshikimate-3-phosphate synthase genes of petunia and tomato. Journal of Biological Chemistry *263*, 4280-4287.

GenTech Seeds (2019). Herbicide tolerant canola stewardship guide. (Australia: GenTech Seeds Pty Ltd).

Graham, K.G., McCaffery, D.W., and Groves, L.M. (2019). Quality of Australian canola 2018-2019. Report No. 25.

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

GRDC (2015a). GRDC Canola GrowNotes: Southern region. (Grains Research and Development Corporation).

GRDC (2015b). GRDC Canola GrowNotes: Western region. (Grains Research and Development Corporation).

GRDC (2017a). GRDC Canola GrowNotes: Northern. (Grains Research and Development Corporation).

GRDC (2017b). GRDC GrowNotes: Herbicide Use (Grains Research and Development Corporation).

GRDC (2019). Intergrated wed management in Australian cropping systems. (Australia: Grains Research and Development Corporation).

Gressel, J. (2002). Molecular biology of weed control (New York, USA: Taylor & Francis).

Groves, R.H., Hosking, J.R., Batianoff, G.N., Cooke, D.A., Cowie, I.D., Johnson, R.W., Keighery, G.J.*, et al.* (2003). Weed categories for natural and agricultural ecosystem management (Bureau of Rural Sciences, Canberra).

Hall, L., Topinka, K., Huffman, J., Davis, L., and Good, A. (2000). Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers. Weed Science *48*, 688-694.

Harker, K.N., Clayton, G.W., Blackshaw, R.E., O'Donovan, J.T., and Stevenson, F.C. (2003). Seeding rate, herbicide timing and competitive hybrids contribute to integrated weed management in canola (*Brassica napus*). Canadian Journal of Plant Science *83*, 433-440.

Harrington, D. (2012). Forage brassicas - a viable alternative. (Grains Research and Development Corporation).

Hartley, R.W. (1988). Barnase and barstar, expression of its cloned inhibitor permits expression of a cloned ribonuclease. Journal of Molecular Biology *202*, 913-915.

Hartley, R.W. (1989). Barnase and barstar: two small proteins to fold and fit together. Trends in Biochemical Sciences *14*, 450-454.

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

Heap, I. (2020). The International Survey of Herbicide Resistant Weeds. (Available online, accessed 14 December 2020).

Heenan, P.B., Goeke, D.F., Houliston, G.J., and Lysak, M.A. (2012). Phylogenetic analyses of ITS and *rbcL* DNA sequences for sixteen genera of Australian and New Zealand Brassicaceae result in the expansion of the tribe Microlepidieae. Taxon *61*, 970-979.

Heritage Seeds (2016). Brassica and summer forage crop guide.

Hérouet, C., Esdaile, D.J., Mallyon, B.A., Debruyne, E., Schulz, A., Currier, T., Hendrickx, K.*, et al.* (2005). Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the *pat* and *bar* sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. Regulatory Toxicology and Pharmacology *41*, 134-149.

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

Invasive Plants and Animals Committee (2015). Noxious weed list for Australian states and territories.

Keese, P. (2008). Risks from GMOs due to horizontal gene transfer. Environmental Biosafety Research *7*, 123-149.

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

Kirkegaard, J.A., Sprague, S.J., Dove, H., Kelman, W.M., Marcroft, S.J., Lieschke, A., Howe, G.N.*, et al.* (2008). Dual-purpose canola - a new opportunity in mixed farming systems. Australian Journal of Agricultural Research *59*, 291-302.

Klaassen, C.D., and Watkins, J.B., eds. (2010). Casarett & Doull's Essentials of Toxicology, 2nd edn (New York: McGraw-Hill).

Knispel, A.L., McLachlan, S.M., Van Acker, R.C., and Friesen, L.F. (2008). Gene flow and multiple herbicide resistance in escaped canola populations. Weed Science *56*, 72-80.

Krebbers, E., Seurinck, J., Herdies, L., Cashmore, A.R., and Timko, M.P. (1988). Four genes in two diverged subfamilies encode ribulose-1,5-bisphosphate carboxylase small subunit polypeptides of *Arabidopsis*. Plant Molecular Biology *11*, 745-759.

Lefol, E., Danielou, V., and Darmency, H. (1996). Predicting hybridization between transgenic oilseed rape and wild mustard. Field Crops Research *45*, 153-161.

Liu, Y., Wei, W., Ma, K., Li, J., Liang, Y., and Darmency, H. (2013). Consequences of gene flow between oilseed rape (*Brassica napus*) and its relatives. Plant Science *211*, 42-51.

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

MacDonald, R.L., and Kuntz, G.J. (2000). Monitoring program to assess the occurrence and fate of SeedLink canola volunteers following the 1999 growing season on Western Canada. Report No. AC00-03. (Aventis CropScience).

Mailer, R. (2004). Canola meal - limitations and opportunities. (Australian Oilseed Federation. Available online).

Mariani, C., De Beuckeleer, M., Truettner, J., Leemans, J., and Goldberg, R.B. (1990). Induction of male sterility in plants by a chimaeric ribonuclease gene. Nature *347*, 737-738.

Mariani, C., Gossele, V., De Beuckeleer, M., De Block, M., Goldberg, R.B., De Greef, W., and Leemans, J. (1992). A chimaeric ribonuclease-inhibitor gene restores fertility to male sterile plants. Nature *357*, 384-387.

Matthews, P., McCaffery, D., and Jenkins, L. (2020). Winter crop variety sowing guide. (NSW Department of Primary Industries).

Meffin, R., Duncan, R.P., and Hulme, P.E. (2018). Testing weed risk assessment paradigms: Intraspecific differences in performance and naturalisation risk outweigh interspecific differences in alien *Brassica*. Journal of Applied Ecology *55*, 516-525.

Meier, U., Bleiholder, H., Buhr, L., Feller, C., Hack, H., Heß, M., Lancashire, P.D.*, et al.* (2009). The BBCH system to coding the phenological growth stages of plants–history and publications. Journal für Kulturpflanzen *61*, 41-52.

Michiels, F., Botterman, J., and Cornelissen, M. (1996). Method to obtain male-sterile plants. Patent No. WO 96/26283.

Monsanto Company (2010). Molecular analysis of glyphosate-tolerant Roundup Ready® 2 (RR2) canola MON 88302. Monsanto Study Report, MSL0022523. (Monsanto Company (unpublished).).

Monsanto Company (2011). Phenotypic evaluation and environmental interactions of glyphosate tolerant canola MON 88302 in U.S. and Canadian field trials during 2009. Monsanto Study Report, MSL0022875. (Monsanto Company (unpublished).).

Murakami, T., Anzai, H., Imai, S., Sathah, A., Nagaoka, K., and Thompson, C.J. (1986). The bialaphos biosynthetic genes of *Streptomyces hygroscopicus*: molecular cloning and characterisation of the gene cluster. Molecular and General Genetics *205*, 42-50.

Ng, K., Raphael, B., Maloney, L., Evans, J., and Parsons, S. (2021). The state of weeds data collection in Australia. (Canberra, Australia: Australian Bureau of Agricultural and Resource Economics and Sciences).

Norton, R. (2003). A survey of roadside canola. Paper presented at: 13th Australian Research Assembly on Brassicas (Tamworth NSW, Australia).

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

OECD (1999a). Consensus document on general information concerning the genes and their enzymes that confer tolerance to glyphosate herbicide. Report No. ENV/JM/MONO(99)9. (Organisation for Economic Cooperation and Development).

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

OECD (2002). Series on Harmonization of Regulatory Oversight in Biotechnology, No 25. Module II: Phosphinothricin. Report No. ENV/JM/MONO(2002)14. (Organisation for Economic Cooperation and Development).

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

OECD (2012). Consensus document on the biology of the Brassica crops. (Organisation for Economic Cooperation and Development).

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

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

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

OGTR (2021). Risk Assessment and Risk management plan for DIR 175: Commercial release of canola (*Brassica napus*) genetically modified for herbicide tolerance and a hybrid breeding system (MS11). (Canberra, Australia: Office of the Gene Technology Regulator).

Padgette, S.R., Barry, G.F., Re, D.B., Eichholtz, D.A., Weldon, M., Kolacz, K., and Kishore, G.M. (1993). Purification, cloning and characterisation of a highly glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp.strain CP4 [Unpublished]. Report No. MSL:12738. (Monsanto Company, Saint Louis, Missouri, USA).

Padgette, S.R., Kolacz, K.H., Delannay, D.B., Re, D.B., LaVallee, B.J., Tinius, C.N., Rhodes, W.K.*, et al.* (1995). Development, identification, and characterization of a glyphosate-tolerant soybean line. Crop Science *35*, 1451-1461.

Padgette, S.R., Re, D.B., Barry, G.F., Eichholtz, D.E., Delannay, X., Fuchs, R.L., Kishore, G.M.*, et al.* (1996). New weed control opportunities: development of soybeans with a Roundup Ready gene. In Herbicide-resistant crops: agricultural, environmental, economic, regulatory and technical aspects, S.O. Duke, ed. (Boca Raton: CRC Press), pp. 53-84.

Perez-Prat, E., and van Lookeren Campagne, M.M. (2002). Hybrid seed production and the challenge of propagating male-sterile plants. Trends in Plant Science *7*, 199-203.

PGG Wrightson Seeds (2020). Brassica management guide. (PGG Wrightson Seeds (Australia) Pty Ltd,).

Phillips, B.B., Williams, A., Osborne, J.L., and Shaw, R.F. (2018). Shared traits make flies and bees effective pollinators of oilseed rape (*Brassica napus* L.). Basic and Applied Ecology *32*, 66-76.

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

Roadside Conservation Committee (2014). Roadside Environmental Weeds List. (Department of Parks and Wildlife, Western Australia).

Rouan, D., and De Both, G. (2018). Hybrid *Brassica* plants and methods for producing same. Patent No. US 9,920,332 B2 (USA).

Salisbury, P.A. (2002). Genetically modified canola in Australia: agronomic and environmental considerations (Australian Oilseed Federation, Melbourne, Australia).

Schafer, M.G., Ross, A.A., Londo, J.P., Burdick, C.A., Lee, E.H., Travers, S.E., Van de Water, P.K.*, et al.* (2011). The establishment of genetically engineered canola populations in the U.S. PLOS ONE *6*, e25736.

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

Schulz, A., Kruper, A., and Amrhein, N. (1985). Differential sensitivity of bacterial 5-enolpyruvyl-shikimate-3-phosphate synthases to the herbicide glyphosate. FEMS Microbiology Letters *28*, 297-301.

Seed Services Australia (2013). Seed certification manual. (Urrbrae, Australia: Division of Primary Industries & Resources South Australia (PIRSA)).

Shackley, B., Paynter, B., Troup, G., Bucat, J., Seymour, M., and Blake, A. (2019). 2020 Western Australian crop sowing guide. (Grains Research and Development Corporation).

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

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

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

Storrie, A. (2018). The challenges of herbicide resistance in non-agricultural weed management systems. In 21st Australasian Weeds Conference (Sydney, Australia: The Weed Society of New South Wales Inc.).

Sutherland, S. (1999). Canola Weed Management. (Australian Oilseeds Federation).

Sweet, J.B. (1999). Monitoring the impact of releases of genetically modified herbicide tolerant oilseed rape in the UK. In Methods of Risk Assessment of Transgenic Plants III Ecological risks and propspects of transgenic plants, K. Ammann, Y. Jacot, V. Simonsen, and G. Kjellsson, eds. (Basel, Switzerland: Birkhäuser Verlag), pp. 159-169.

Thompson, C.J., Movva, N.R., Tizard, R., Crameri, R., Davies, J., Lauwereys, M., and Botterman, J. (1987). Characterization of the herbicide-resistance gene *bar* from *Streptomyces hygroscopicus*. EMBO Journal *6*, 2519-2523.

Ulyanova, V., Vershinina, V., and Ilinskaya, O. (2011). Barnase and binase: twins with distinct fates. The FEBS Journal *278*, 3633-3643.

USDA-APHIS (1999). Response to AgrEvo petition 98-278-01p for determination of nonregulated status for canola transformation events MS8 and RF3 genetically engineered for pollination control and tolerance to glufosinate herbicide. (Available online).

USDA-APHIS (2013). USDA-APHIS 2013 Monsanto Petition (11-188-01p) for Determination of Non-regulated Status of MON 88302 Canola - Plant Pest Risk Assessment. (Animal and Plant Health Inspection Service, United States Department of Agriculture).

USDA-APHIS (2017). Final extended determination of nonregulated status for Bayer CropScience LLP MS11 canola. (Animal and Plant Health Inspection Service, United States Department of Agriculture).

Velten, J., and Schell, J. (1985). Selection-expression plasmid vectors for use in genetic transformation of higher plants. Nucleic Acids Research *13*, 6981-6998.

Warwick, S.I., Francis, A., and Gugel, R.K. (2009). Guide to Wild Germplasm of Brassica and Allied Crops (tribe Brassiceae, Brassicaceae) 3rd Edition (Agriculture and Agri-Food Canada).

Warwick, S.I., Légère, A., Simard, M.J., and James, T. (2008). Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy *Brassica rapa* population. Molecular Ecology *17*, 1387-1395.

Warwick, S.I., Simard, M.J., Légère, A., Beckie, H.J., Braun, L., Zhu, B., Mason, P*., et al.* (2003). Hybridization between transgenic *Brassica napus* L. and its wild relatives: *Brassica rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Erucastrum gallicum* (Willd.) O.E. Schulz. Theoretical and Applied Genetics *107*, 528-539.

Western Australian Herbarium (1998–). FloraBase—the Western Australian Flora. (Available online, accessed 25 November 2020: Department of Biodiversity, Conservation and Attractions).

Yang, W. (2011). Nucleases: diversity of structure, function and mechanism. Quarterly Reviews of Biophysics *44*, 1-93.

Zand, E., and Beckie, H.J. (2002). Competitive ability of hybrid and open-pollinated canola (*Brassica napus*) with wild oat (*Avena fatua*). Canadian Journal of Plant Science *82*, 472-480.

Appendix A: Summary of submissions on matters relevant to preparation of the consultation RARMP

The Regulator received several submissions from prescribed experts, agencies and authorities[8] 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 those matters identified by the office (potential for toxicity, allergencity, weediness and harm as a result of gene flow to other canola) should be considered when preparing the RARMP. Recommends that the following specific topics should be addressed in the risk assessment: • potential toxicity of the GM canola, including whether there is buildup of the expressed proteins in response to herbicide application and whether this could affect toxicity • the effect of the commercial release of canola with a hybrid breeding system trait on bee populations • the potential for gene flow to known weeds such as Buchan weed • alternative herbicides from different mode of action groups that would be effective on the GMOs.	The potential toxicity of the GM canola lines, including whether there is buildup of the expressed proteins in response to herbicide application and any effect on toxicity is addressed in Chapter 2, Section 2.4.1 (Risk scenario 1). The effect of the hybrid breeding system (MS11 x RF3) on pollen production in the two GM canola lines proposed for commercial release is discussed in Chapter 1, Section 5.1.2. The parental line MS11 and intermediate breeding line MS11 x MON 88302 (Chapter 1, Section 2) do not produce pollen, but are only grown in small amounts for generating the GMOs. The GMOs themselves are fully fertile (Section 6.1). The potential for gene flow to known weeds such as Buchan weed is addressed in Chapter 1, Section 7.3.1 and Chapter 2, Section 2.4.5 (Risk Scenario 5). Alternative herbicides from different mode of action groups that would be effective on the GMOs are addressed in Chapter 2, Section 2.4.2 (Risk scenario 2).
	Recommends that the Regulator should consider the expression and function of the *barstar* gene in the MS11 line and any implication for the risk assessment.	The expression and function of the *barstar* gene in the MS11 line and any implication for the risk assessment are discussed in Chapter 1, Sections 5.1.2, 5.2.3 and 6.2.2, and Chapter 2, Section 2.4.1 (Risk scenario 1).
2	Nil response as Shire does not have Agribusinesses.	Noted.
3	Trusts the scientific basis will have no environmental risks to the area as Council does not have the expertise to comment on the proposal.	Noted.

[8] 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	As most of the community are farmers or farming related and currently use GM seed for their cropping, the general consensus is that councilors understand the benefits of this technically to improve production and reduce cost.	Noted.
5	While consultation is appreciated, Council does not engage, nor have access to, advice that would assist in responding to this matter. Trusts that the State and Federal Government agencies that may be involved in the consideration of this application will undertake the due diligence necessary to ensure the activity can occur without any substantive negative offsite impacts to human health or the environment or locality. Council will record the application in its system and would appreciate notice of the assessment outcome.	Noted.
6	Council are not the subject matter experts in this area and unable to provide advice as requested.	Noted.
7	Overall, the application has negligible risks to the health and safety of people and the environment. Has no additional concerns to be investigated beyond the usual risks identified during the development of a RARMP. Notes that there will be further opportunity for input into DIR 178 once the RARMP is made available for comment.	Noted.
8	Has reviewed this application and offers no specific feedback. Notes that as per the ACT Gene Technology (GM Crop Moratorium) Act 2004, there are currently 2 Moratorium Orders that prohibits the use, release and propagation of the following introduced genes of genetically modified canola in the ACT: • *Streptomyces hygroscopicus* • *Bacillus amyloloquefaciens* Therefore, if a licence is granted for DIR 178, then the use of these genetically modified genes would remain prohibited within the ACT.	Noted.
9	As this licence application is for a commercial release involving conventional breeding techniques to produce the stacked events, the members thought it is important for the OGTR	The potential for development of herbicide resistance in GM canola with stacked genes for multiple herbicide tolerance and management of GM canola with multiple

Submission	Summary of issues raised	Comment
	to consider during preparation of the RARMP the following issues: • Long-term management strategies to minimise development of herbicide resistance in GM canola with stacked genes for multiple herbicide resistance eg: the DIR 178 canola • Expand on strategies to control the herbicide tolerant GM canola volunteers with stacked genes for multiple herbicide resistance.	herbicide tolerance are discussed in Chapter 2, Section 2.4.4 (Risk Scenario 4). As this application is for commercial release, no specific control measures to contain the GM canola lines are proposed in the licence. Management of herbicide tolerant GM canola is outside the remit of the OGTR. APVMA is responsible for assessing the risks of herbicide use, and registration of the formulations and use patterns of the herbicides on herbicide tolerant GM canola, including any restrictions and mitigation measures suitable for conditions in Australia. The industry is responsible for development of management strategies to control herbicide tolerant GM canola volunteers.
	Overall, the members supported the licence application of the BASF Australia Ltd.	Noted.
10	It is noted that: MS11 (glufosinate tolerance) is currently under evaluation as part of BASF's commercial release application DIR 175/2021; RF3 has been authorised for commercial release under the licence DIR 021/2002; and MON 88302 (glyphosate tolerant canola) has been authorised for commercial release under licence DIR 127/2014. Previous Risk Assessment and Risk Management Plans (RARMPs) on the individual GM canola lines (DIR 175/2021 - MS11, DIR 021 /2002 - RF3, DIR 127/ 2014 – MON 88302), and on hybrids MON88302 x RF3 (DIR 138/2016) will be relevant to the preparation of the RARMP for this application.	Noted. As discussed in Chapter 2, Section 1, risk scenarios in previous RARMPs prepared for the parental lines and similar GMOs are considered when postulating risk scenarios.
	Given the recent evidence of canola dispersal and weediness, it is recommended the following be included in the RARMP: Information on seed dispersal and on factors such as increased abiotic stress tolerance that may impact seed survival, persistence and weediness in natural ecosystems. • It is noted that previous RARMPs have assessed the risk of pollen and seed dispersal and gene transfer to non-GM canola and weedy relatives and potential environmental harm (i.e. weediness) and any data for these factors from the individual GM lines MS11, RF3, and MON88302 should be used in this RARMP.	The introduced genes for a hybrid breeding system or herbicide tolerance do not obviously contribute to increased abiotic stress tolerance in the GM canola lines proposed for release. However, these factors are considered and discussed in the Chapter 2, Section 2.4.3 (Risk scenario 3). Weediness of the parental GM canola lines is discussed in Chapter 1, Section 5.3.
	• It is recommended that data be included to support conclusions regarding whether the final stacked GM canola will or will	Relevant data have been included in Chapter 1, Section 6.2.3, which support the conclusion that the stacked GM canola line is

Submission	Summary of issues raised	Comment
	not differ from the non-GM canola in characteristics that may impact weediness.	comparable to non-GM canola in characteristics relevant to weediness.
	Further discussion regarding seed dispersal and weediness: The RARMP should discuss GM canola seed dispersal by wind as the primary route of dispersal to natural ecosystems • Pollen and seed dispersal are routinely assessed in RARMPs as potential pathways to harm i.e. weediness. It is recommended that the RARMP discuss that canola seed dispersal by wind can be problematic due to seed pod shattering, large seed numbers and the very small size of seeds. The RARMP should also note recent evidence that, while seed spillage is the prime seed dispersal route, small seed size and shattering are identified as a spontaneous dispersal route of canola seed in Canada. • For a commercial release, seed dispersal by wind may be a significant pathway for release into the environment.	Canola seed dispersal by wind and shattering as a dispersal route is discussed in Chapter 2, Section 2.4.2 (Risk scenario 2) and Section 2.4.3 (Risk scenario 3).
	The RARMP should discuss data on potential increased stress tolerance in GM canola. • Environmental harm could be caused directly by the trait or indirectly by the trait conferring weediness characteristics. For example, recent genomics assessment of stacked versus single trait herbicide tolerant soybean showed that the stacked variety had increased levels of genes associated with abiotic stresses. • While direct adverse environmental harm by the traits of herbicide tolerance are considered unlikely, the RARMP should discuss whether there are any indications or data regarding whether the stacked trait for herbicide tolerance changes abiotic stress tolerance.	Data collected from field trials in Canada and the USA that are relevant to the assessment for any potential for changed abiotic stress tolerance of the GM canola lines are included and discussed in Chapter 1, Section 6.2.3.
	It is recommended that the RARMP discuss the similarity of environments for the acceptance of US and Canadian data on GM canola that will be grown in Australia, especially in light of the differences observed in Australian field trial data and field trials in Canada and the US.	Transferability of field trial data from Canada and the USA for this application is discussed in Chapter 1, Section 6.2.3.
	While canola is not currently a significant weed of natural environments in Australia, largely because of its intolerance to abiotic (lack of	Weediness of canola in agricultural areas is discussed in Chapter 1, Section 4.2 and Chapter 2, Section 2.4.2 (Risk Scenario 2).

Submission	Summary of issues raised	Comment
	water, drought) and biotic stresses (diseases), it is a significant weed of agricultural areas. GM HT canola has emerged as a significant weed (of agricultural areas) in Argentina and Canada. It is also noted that crop transgenes have moved into truly wild populations for only three GM crops, one of which is HT canola.	Movement of transgenes from HT canola to weed populations is discussed in Chapter 2, Section 2.4.5.

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

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

Submission	Summary of issues raised	Comment
1	Town has no farming or canola growing area in its jurisdiction and therefore has no official policy on GM canola but would like this to be undertaken in a way that is safe to both the public and the environment.	Noted.
2	Council raises no issues with the commercial release of the product.	Noted.
3	Agrees that all plausible risk scenarios have been identified and no additional relevant information was identified. Agrees with the overall conclusion of the RARMP.	Noted.
4	Have some general concerns over using GMOs in agriculture that include: • Causing resistant pests and weeds • GM crops can spread and the introduced genes can be transferred to their wild-type counterparts, causing unintended harm to the environment • Genetically engineering food is a relatively new practice, little is known about the long-term effects and safety • Effects of long-term use of herbicides and their subsequent residues on soil health, plant fecundity, bee populations and groundwater • The introduced genes may have unknown consequences to change the organism's metabolism, growth rate, and/or response to external environmental factors and cause harm to natural environment.	The RARMP concluded that the commercial release of the GM canola lines poses negligible risks to the health and safety of people and the environment. FSANZ is responsible for human food safety assessment, including GM food. Issues relating to herbicide use are outside the scope of the Regulator's assessments. The APVMA has regulatory responsibility for the registration of agricultural chemicals, including herbicides.
	Does not believe that GMO food is needed to feed the entire world population as GM crops may have reduced yield. Suggests that there are other ways to solve the issue of food insecurity, such as improving crops through	Matters relating to choice of farming systems is outside the scope of the Regulator's assessment required by the Act.

Submission	Summary of issues raised	Comment
	symbiotic relationships with diverse microorganisms.	
	Suggests that Australia should become a signatory of the *Cartagena Protocol on Biosafety to the Convention on Biological Diversity 2003*.	This matter is outside the scope of the Regulator's assessment.
	Mentions some research works for expression, control and detection of engineered genes that can be applied to minimise potential risks and suggests that if used wisely GMOs can result in an improved economy without doing more harm than good.	Choice of technology by researchers and developers is outside the scope of responsibility of the Regulator, as are social-economic considerations. The Regulator assesses the application as received, for potential harm to the health and safety of people and the environment.
5	Reviewed this application and has no objections to the licence being issued. Also notes that the commercial release will mean the use of this canola in human and animal feed but not aware of any credible scientific evidence that would give rise to concerns for human or animal health at this stage.	Noted.
6	Accepts that overall BASF's application has negligible risks to the health and safety of people and the environment, and satisfied that the measures taken to manage the short and long term risks from the proposal are adequate.	Noted.
7	The draft licence conditions seem appropriate and commensurate with the level of risk that this commercial release may pose. Notes that no new work has been undertaken in recent times on rates of outcrossing to other canola crops or Brassica weeds (except the published work of Rieger et al. 2002) but believes that is because outcrossing has not been a problem. Overall, supports the Regulator's conclusion that this release poses negligible risk of harm to human health and the environment.	Noted.
8	Comments that there remains some uncertainty regarding the adequacy of overseas confined field trial (CFT) data to draw a conclusion on abiotic and biotic stress tolerance in the stacked GM canola lines. An unintended increase in abiotic or biotic stress tolerances could lead to increased survival or weediness if it were to escape to natural environments or if gene transfer to weedy relatives in Australia occurred.	
	Therefore, recommends including additional information in the RARMP to support the	Canola is cultivated across a range of agro-ecological zones in Australia. Field trial data

Submission	Summary of issues raised	Comment
	assessment that there is no greater potential for increased stress tolerance in the stacked GM canola to be grown in Australia: • Discuss the similarity of environments (and provide supporting evidence) for the acceptance of US and Canadian CFT data on abiotic and biotic stress tolerance of GM canola. Stating that environments are similar because they both contain arid areas may not be sufficient basis for concluding on comparability. • Information on the transportability of overseas field trial data on GM canola compared to other GM crops. Cites an article in support of the view that transportability is higher for highly domesticated crops such as cotton or corn that do not require strict similarity of zones and do not have weedy potential.	related to characteristics and environmental interactions were collected from trial sites selected from the agro-ecological zones that cover both rain-fed and irrigated cropping areas in Canada and the USA. Some of the Australian agro-ecological zones have very similar climate with that of the selected trial sites in Canada and the USA. Australia has lengthy experience with both GM and non-GM canola cultivation. A substantially similar stack (MS8 × RF3 × MON 88302) to that considered here (MS11 × RF3 × MON 88302) was approved in 2016 for commercial release in Australia under DIR 138, the difference being the inclusion of the *barstar* gene in the MS11 event. The phenotypic data for that release was also based on overseas CFTs and no increase in abiotic or biotic stress tolerances that could lead to increased survival or weediness was identified. To further clarify this issue, additional discussion has been included in Chapter 1, Section 6.2.3 and some additional text on uncertainty included in Chapter 2, Section 3.
	• Cites recent information on changes in fitness traits for glyphosate tolerant GM plants and unintended changes in abiotic and biotic stress tolerance gene expression in stacked versus single transgene GM plants.	Additional information on potential for unintended changes in fitness resulting from *epsps* gene overexpression in GM plants has been included in Chapter 2, Section 2.4.3 (RS3). Changes in gene expression profile highlighted through transcriptomic analysis are not unexpected during plant breeding, whether GM or conventional (see for example Liu et al. (2020) Plant J 103(6): 2236). Comparative change at the phenotypic level is of more note and the risk assessment considers the potential for harm to human health and the environment from any such changes.

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

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

Submission	Summary of issues raised	Comment
1	Does not want to eat any GM food. Any food using this GM product must be labelled so people can choose not to buy. Asks "Why are you allowing greedy farmers to dictate what people can eat and feed people poison while making them richer with larger harvests".	Food Standards Australia New Zealand (FSANZ) has regulatory responsibility for food safety assessments and food labelling, including of GM food. Products derived from GM canola lines included in this application have been approved by FSANZ for use in human food. More information about their assessments is available from the FSANZ website. The RARMP concludes that the commercial release of the GM canola poses negligible risks to the health and safety of people. Choices of farming systems and crops and any associated socio-economic impacts are outside the scope of the Regulator's assessment required by the Act. These issues are the responsibility of the States and Territories, and industry.
	States that this country has become disgusting by following the American style of capitalism and suggests following Europe in preference.	Noted.
2	Asks if this is the first time herbicide resistant GM canola will be used for human food in Australia and which other herbicide and insecticide resistant GM crops have been used for human food in Australia.	Relevant information can be found from the FSANZ website.
	Comments that the herbicides were not listed in the public notification.	The public notification includes a link to the OGTR Website, where Q&As and the RARMP for DIR 178 list the herbicides as glyphosate and glufosinate.
3	On behalf of the organic industry of Australia, strongly objects to this decision on GM canola based on the following claims: Adventitious contamination of organic crops and the loss of organic certification that will certainly happen, as in Western Australia. This would eventually destroy the organic status of all organic canola farmers and organic processors using canola in their final products for Australian and international markets. All international markets would be lost causing big losses in export income to Australia.	When deciding whether or not to issue a licence, matters that relate to marketing and trade, including coexistence of GM and non-GM crops, are outside the legislative responsibility of the Regulator. These are matters for State and Territory governments, who may designate GM free zones for marketing purposes. The legal case and incidences in WA with commercially approved GM crops related to segregation and marketing issues, not health and safety issues, and as such is

Submission	Summary of issues raised	Comment
	In contrast to organic farmers, chemical farming industry has long been involved in environmental vandalism and now they want to pollute food. Asserts that chemical farming industry wants to get rid of organic farming so they can increase their price. This decision will cripple many organic farming families and exporters and destroy the clean image that Australia has in the world.	outside the scope of the Regulator's assessment required by the Act.
	Has concerns that once the GM canola lines and their products enter general commerce, these products will not be labelled. Suggests labelling all GM produce to allow consumer choice and let the marketplace decide the future of GM in our food.	See comments for Submission 1 regarding issues relating to food safety and labelling.
4	Supports the adoption of new technology in farming systems for the needs of farmers in managing the Australian farming environment. GM canola has been grown successfully since 2008 in Australia and grain producers have been able to meet expectation of markets and regulatory concerns. No concerns for this release in relation to the two specific chemical resistant traits. They have been used globally for some years including as a double stacked trait. As such the GM canola offers farmers greater options to manage herbicide resistance. Therefore, supports the application DIR 178.	Noted.