Australian Government
Department of Health
Office of the Gene Technology Regulator

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)

Applicant: BASF Australia Ltd

May 2021

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application DIR 175

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 175
Applicant	BASF Australia Ltd (BASF)
Project title	Commercial release of canola (*Brassica napus*) genetically modified for herbicide tolerance and a hybrid breeding system (MS11)[1]
Parent organism	*Brassica napus* L. (canola)
Introduced genes and modified traits	**One gene for herbicide tolerance:** • *bar* gene from *Streptomyces hygroscopicus* for glufosinate 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 use as a parent line 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 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.

[1] The title of the application submitted by BASF is "Commercial release of canola (*Brassica napus*) genetically modified for herbicide tolerance and a hybrid breeding system".

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; proteins similar to the introduced proteins are widespread in the environment; the GM canola was licenced for field trials in Australia between 2011 and 2017 with no reported adverse or unexpected effects; the male sterility trait reduces the ability of the GM canola to spread and persist, compared with non-GM canola; and the GM canola has limited capacity to survive in natural habitats. In addition, food made from the GM canola has been assessed and approved by Food Standards Australia New Zealand as safe for human consumption.

Risk management

The risk management plan concludes that risks from the proposed dealings can be managed 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*
AFSI	Agriculture and Food Systems Institute
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
BLAST	Basic Local Alignment Search Tool
bp	Base pair
BC1, etc.	First backcross generation
CANBR	Centre for Australian National Biodiversity Research
CCI	Confidential Commercial Information under section 185 of the *Gene Technology Act 2000*
CMP	Crop management plan
DAWE	Department of Agriculture, Water and the Environment
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic acid
DW	Dry weight
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
F1	First hybrid generation
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Global Food and Agriculture Statistics of FAO
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)
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
T2, etc.	Second generation after transformation
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
WT	Wild type

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.

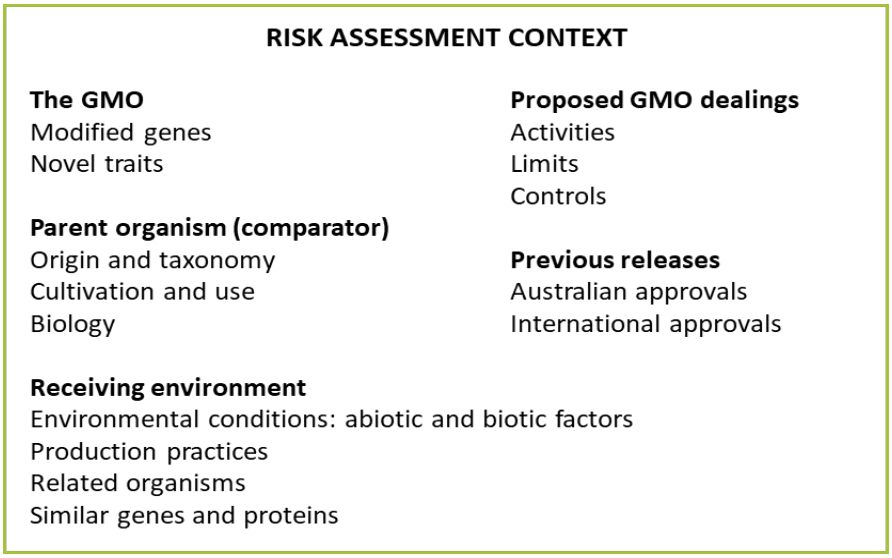

Figure 1 Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.

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. Two 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 a genetically modified (GM) canola line (MS11), which contains two introduced genes that form part of a hybrid breeding system and one introduced gene that confers herbicide tolerance. This event is also known by the unique OECD identifier BCS-BNØ12-7.

14. The applicant is seeking approval for the release to occur Australia-wide, subject to any moratoria imposed by States and Territories for marketing purposes. MS11 canola 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.

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

[2] BASF is seeking approval for unrestricted commercial release of MS11 canola 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.

(a) conduct experiments with the GMO

(b) breed the GMO

(c) propagate the GMO

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

(e) grow the GMO

(f) import the GMO

(g) transport the GMO

(h) dispose of the GMO

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

Section 3 The parent organism

16. 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).

17. *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).

18. *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).

19. *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.

20. 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).

21. 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.

22. 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.

23. 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.

3.1 Canola as a crop

24. 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).

25. 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).

26. 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 5 (the receiving environment).

3.2 Weed risk potential for canola outside cultivation

27. *Brassica napus* is not recorded in the *Weeds of National Significance* list (DAWE website, accessed July 2020), the *National Environmental Alert List* (DAWE website, accessed July 2020) or the Noxious Weed List for Australian States and Territories (Invasive Plants and Animals Committee, 2015).

28. 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 3.2.1 to 3.2.3, below.

3.2.1 Potential to cause harm

29. 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, and
- moderate potential to act as a reservoir for pests or pathogens (OGTR, 2017).

30. *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).

31. 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).

3.2.2 Invasiveness

32. 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).

3.2.3 Actual distribution

33. 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).

34. 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).

35. 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).

36. 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).

3.2.4 Management of volunteer canola

37. Canola volunteers generally emerge in the year following a canola crop, but may emerge for up to three years (Australian Oilseeds Federation, 2019), with the seedbank declining rapidly (Baker and Preston, 2008).

38. 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 ammonium), 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 4 The GM canola – nature and effect of genetic modification

4.1 The genetic modification

4.1.1 Details of the introduced genetic elements

39. The genes and regulatory sequences introduced into MS11 canola are listed in Table 1. The three introduced genes are from soil-borne bacteria (*Streptomyces hygroscopicus* and *Bacillus amyloliquefaciens*). Short regulatory sequences that control expression of the introduced genes are derived from plants (thale cress, *Arabidopsis thaliana*; and tobacco, *Nicotiana tabacum*) and from soil-borne bacteria (*Agrobacterium tumefaciens* and *B. amyloliquefaciens*).

40. The *barnase/barstar* hybrid breeding system genes and the *bar* herbicide tolerance gene have been previously assessed and approved for release in Australia (see Section 6.1). The novel trait in MS11 canola is expression of the *barstar* fertility restoration gene by a weak promoter in the male sterile line (see Section 4.2.2).

41. Although some of the introduced regulatory sequences in MS11 canola are derived from a plant pathogen (*Agrobacterium tumefaciens*) or a toxic plant (tobacco), by themselves they do not cause disease or toxicity. The regulatory elements present in MS11 canola have been previously assessed by Australian and international regulators without identifying an increase in risk compared with endogenous regulatory elements in canola.

Table 1 Introduced genes and regulatory elements in canola line MS11

Genetic element	Source	Encoded protein	Function
PssuAt	*Arabidopsis thaliana*	-	*bar* promoter
bar	*Streptomyces hygroscopicus*	phosphinothricin N-acetyltransferase (PAT)	Gene conferring glufosinate ammonium tolerance
3'g7	*Agrobacterium tumefaciens*	-	*bar* terminator
Pta29	*Nicotiana tabacum*	-	*barnase* promoter
barnase	*Bacillus amyloliquefaciens*	barnase (ribonuclease)	Gene conferring male sterility
3'barnase	*B. amyloliquefaciens*	-	*barnase* terminator
3'nos	*A. tumefaciens*	-	*barnase* terminator
Pnos	*A. tumefaciens*	-	*barstar* promoter
barstar	*B. amyloliquefaciens*	barstar (ribonuclease inhibitor)	Gene conferring fertility restoration*
3'g7	*A. tumefaciens*	-	*barstar* terminator

* The *barstar* gene confers fertility restoration when expressed by Pta29 or similar promoters. In MS11, *barstar* is expressed by a weaker promoter, which does not express the protein at a sufficient level to confer fertility restoration. Low level expression of *barstar* improves transformation efficiency during the development of the GMO (see Section 4.2.2).

4.1.2 Method of genetic modification

42. MS11 canola was generated using *Agrobacterium*–mediated transformation. This method has been widely used in Australia and overseas for introducing genes into plants. More information can be found in the document *Methods of Plant Genetic Modification* on the Risk Assessment References page on the OGTR website.

43. Genetic elements of the transformation plasmid pTCO113 were delivered by *A. tumefaciens* into embryogenic callus induced from dissected hypocotyl segments of canola variety N90-740. Plasmid

pTCO113 contains three expression cassettes between the right and left borders of the transfer DNA (T-DNA) for expression of the *bar*, *barnase* and *barstar* genes. These genes and the regulatory elements controlling their expression (listed in Table 1) were delivered as a single insertion. Genetic elements outside of the left and right borders of the T-DNA (the plasmid backbone) were not transferred (Section 4.3).

4.2 The introduced genes, their encoded proteins and associated effects

4.2.1 The bar *gene and its encoded product*

44. MS11 canola contains the bialaphos resistance (*bar*) gene, isolated from the soil-borne bacterium *Streptomyces hygroscopicus* (Thompson et al., 1987), which was first assessed for commercial release in canola under DIR 021/2002. The PssuAt promoter expresses *bar* in all green tissues of the plant (Krebbers et al., 1988; Rouan and De Both, 2018).

45. The *bar* gene encodes a phosphinothricin acetyltransferase (PAT) protein that confers tolerance to glufosinate ammonium herbicide (Hérouet et al., 2005). Glufosinate ammonium is the active component in a number of Group N herbicides (GRDC, 2017b). 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 *S. hygroscopicus* (Murakami et al., 1986). PAT acetylates glufosinate, converting it to *N*-acetyl-L-glufosinate and rendering it inactive (OECD, 2002).

46. The *bar* gene introduced into MS11 canola was modified by a substitution of two N-terminal codons of the original bacterial gene (see RARMP for DIR 021/2002; Thompson et al., 1987; Rouan and De Both, 2018).

4.2.2 The barnase *and* barstar *genes and their encoded products*

47. MS11 canola contains a male sterility (*barnase*) gene and a fertility restoration (*barstar*) gene, isolated from the soil-borne bacterium *Bacillus amyloliquefaciens* (Hartley, 1988), which were first assessed for commercial release in canola under DIR 021/2002.

48. The male sterility gene is expressed in the tapetum during pollen development, causing pollen to disintegrate (De Block and Debrouwer, 1993). Low levels of expression are also observed in anther vascular tissue. The mRNA polyadenylation signals, which are required for gene expression in plants, are provided by the 3' non-translated region of the nopaline synthase gene (3'nos) from *Agrobacterium tumefaciens* (Depicker et al., 1982; Rouan and De Both, 2018).

49. The fertility restoration gene is expressed constitutively at low levels by the Pnos promoter (Depicker et al., 1982; Michiels et al., 1996). The applicant states that *barstar* 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. Constitutive low expression of *barstar* may mitigate effects of leaky expression of *barnase* in non-tapetal cells. Although the *barnase* 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).

50. The *barnase* gene encodes a ribonuclease protein (barnase or RNase Ba) that is expressed extracellularly by *B. amyloliquefaciens*. The *barstar* gene encodes a protein (barstar) that forms a complex with barnase to inhibit its function. Barstar is expressed intracellularly by *B. amyloliquefaciens* to protect itself from the effect of barnase (Hartley, 1988).

4.2.3 Toxicity and allergenicity of the proteins encoded by the introduced genes

51. FSANZ has approved food derived from MS11 canola expressing PAT, barnase and barstar proteins as safe for human consumption (FSANZ, 2017). An assessment by EFSA did not identify any toxicity or allergenicity concerns from the PAT, barnase and barstar proteins expressed in MS11 canola (EFSA GMO Panel et al., 2020).

PAT protein

52. 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; and
- purified PAT protein was not toxic to mice and rats when administered at high doses in acute toxicity studies.

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

Barnase and barstar proteins

54. Barnase acts as a bacteriocin; 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.

55. Barstar is a ribonuclease inhibitor protein, which does not possess enzymatic activity; but, instead, exerts its action by binding to the barnase enzyme to form an inactive complex.

56. The *barnase–barstar* hybrid breeding system, which encodes barnase and barstar proteins, has been extensively assessed in previous RARMPs for commercial release of GM canola (DIR 021/2002, DIR 108 and DIR 138). 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; and
- feeding studies in rabbits, canaries and broiler chickens have shown that RF x MS canola lines (containing barnase and barstar) are nutritionally equivalent to non-GM canola.

57. FSANZ has approved food derived from a GM canola expressing barnase and barstar proteins as safe for human consumption (ANZFA, 2001; FSANZ, 2017).

Glufosinate herbicide metabolites

58. Herbicide metabolites produced in GM plants expressing PAT, following treatment with glufosinate ammonium, have been discussed 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 and DIR 173). The main points are:

- Glufosinate ammonium 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 ammonium to GM plants expressing PAT, the major residue present is NAG, with lower concentrations of glufosinate ammonium and 3-methyl phosphinico-propionic acid (MPP) (OECD, 2002).
- Following application of glufosinate ammonium to non-GM plants, the major residue is glufosinate ammonium, with a small proportion of MPP (OECD, 2002). *N*-acetyl-L-glufosinate is not present.
- Both NAG and MPP are less toxic than glufosinate ammonium (FAO, 2014).

59. 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).

4.2.4 The hybrid breeding system and inheritance of GM traits

60. Hybrid vigour, or heterosis, is a well-known biological phenomenon, whereby the heterozygous offspring of two homozygous parents exhibit greater growth and yield than either of their parents. 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.

61. The hybrid breeding system that is conferred by expression of the *barnase* and *barstar* genes is derived from the common soil bacterium *B. amyloliquefaciens*. *Barnase* encodes a 110 amino acid (~12 kilodalton) ribonuclease (RNase) called barnase, and *barstar* encodes an 89 amino acid (~10 kDa) RNase inhibitor protein, barstar, which specifically binds to barnase and suppresses its activity (Hartley, 1988, 1989).

62. 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).

63. In MS11 canola, *barnase* 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.

64. In order to maintain the MS11 parental line, the hemizygous MS11 canola line is backcrossed with elite germplasm that does not contain the male sterility (MS) trait (Figure 2a). The progeny inherit the MS11 event in a 1:1 ratio. As the *barnase* gene is linked to the *bar* herbicide tolerance trait, spraying the progeny with glufosinate ammonium herbicide destroys all fully fertile plants, leaving only male-sterile plants (Figure 2b). Hybrid commercial seed is produced by crossing the male-sterile plants with a line containing an effective fertility restoration (RF) trait (Figure 2c).

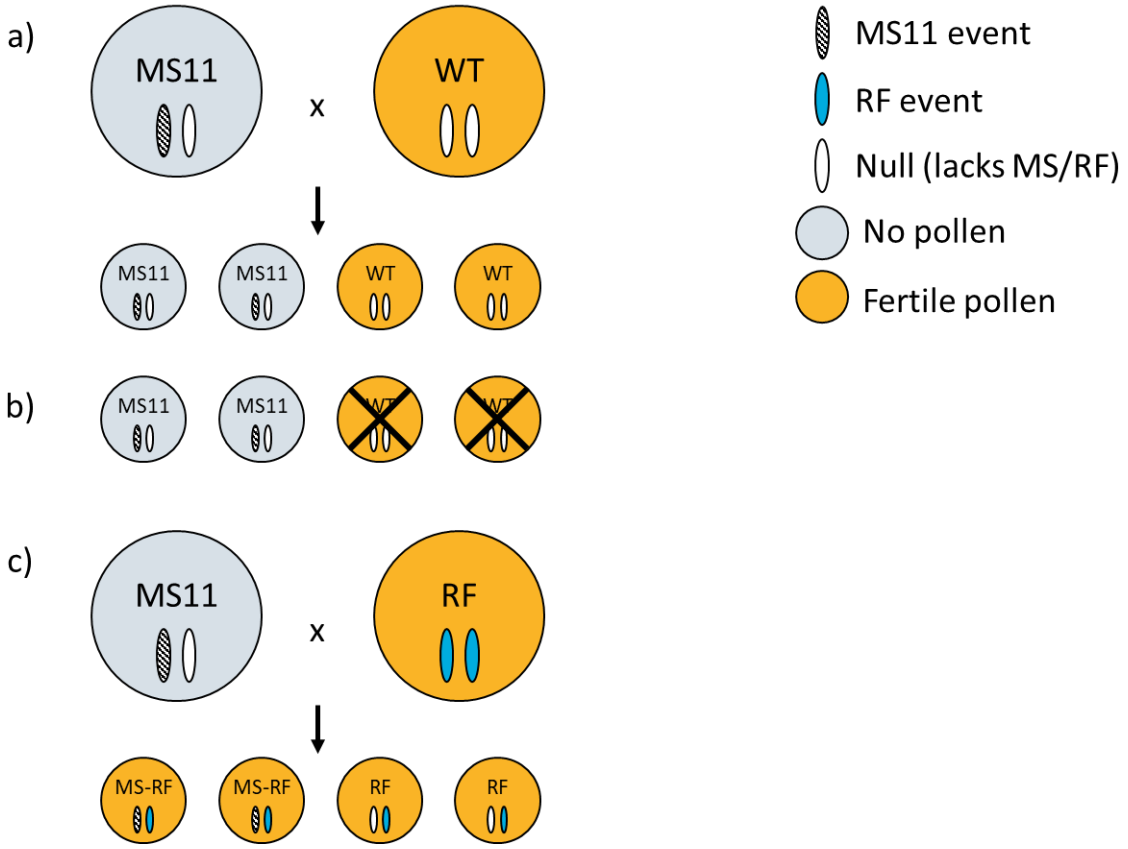

Figure 2 Inheritance of the MS11 trait during maintenance of the parental line and commercial hybrid seed production.
a) Maintenance of MS11 parental line. The hemizygous MS11 line is backcrossed with elite germplasm that does not contain the male sterility (MS) trait (wild type, WT). The MS and herbicide tolerance traits are inherited together in a 1:1 ratio.
b) Spraying progeny with glufosinate ammonium kills male fertile plants.
c) Crossing MS11 canola with a homozygous fertility restoration (RF) line to produce hybrid seed. All progeny have fertile pollen and herbicide tolerance, as the RF line also contains the *bar* gene. Note, dealings with the RF line and the progeny of the MS x RF cross would be authorised under a separate DIR licence.

65. Fertility restoration canola lines contain a *barstar* gene under the control of the same PTa29 promoter sequence as the *barnase* gene in MS lines (Mariani et al., 1992)[3]. This reverses the effect of *barnase* expression in the tapetal cells. Expression of *barstar* has no effect on pollen development, and RF canola plants have a normal appearance and viable pollen. When a MS line containing *barnase* is

[3] The MS11 event also contains a *barstar* gene under the control of a weak promoter. Expression of barstar associated with the MS11 event is not sufficient to restore fertility.

crossed with a RF line containing *barstar*, progeny that inherit both genes display completely normal fertility due to the specific inhibition of barnase activity by barstar (Mariani et al., 1992).

4.2.5 Pollination of male sterile canola plants by sexually compatible species

66. As MS11 canola plants do not produce pollen, they need to be cross-fertilised with pollen from a sexually compatible species in order to set seed. As few as 25 pollen grains per stigma is sufficient to allow seed set in *B. napus*, with 100–200 pollen grains per stigma required for full seed set (Lankinen et al., 2018).

67. Apart from pollen production, other floral traits that influence pollinator visitation are expected to be similar in MS11 canola and wild type *B. napus.*

68. Male sterile flowers are pollinated via insects and wind, with gene flow decreasing with distance from the source pollen. This was recently demonstrated in an experiment conducted by Zhang et al. (2018). Male sterile canola plants were planted at different distances from a small plot of herbicide resistant canola and gene flow was measured (Figure 3). At a distance of 2 m with open pollination, over 32% of male sterile flowers were pollinated by the herbicide resistant plants. At a distance of 128 m with open pollination, only 0.3% of male sterile flowers were pollinated by the herbicide resistant plants. Experiments using insect-proof nets demonstrated that both wind and honeybees mediated a similar level of pollination (Figure 3).

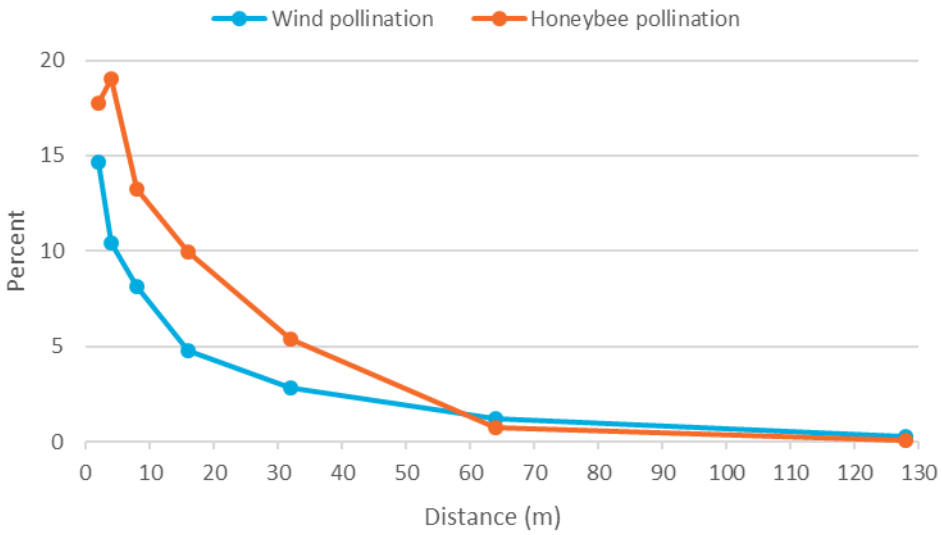

Figure 3 Gene flow rates (%) from herbicide resistant *B. napus* to male sterile *B. napus* via wind and honeybees.
Visualisation of data published in Table 4 of Zhang et al. (2018). $LSD_{0.05}$ (wind pollination), 2.5; $LSD_{0.05}$ (honeybee-mediated pollination), 6.5.

4.3 Characterisation of the GMO

69. BASF provided a number of reports characterising MS11 canola. Certain information in these reports was declared Confidential Commercial Information (CCI) under section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

4.3.1 MS11 insertion locus

70. Sequencing of the insert and flanking regions of the T-DNA insertion in MS11 canola showed that a 40 base pair target site deletion of the *B. napus* genome occurred during integration of the MS11 T-DNA

(Anon., 2008). The 5' flanking sequences (419 bp) and 3' flanking sequences (556 bp) adjacent to the inserted transgenic sequences are completely identical to the 5' and 3' flanking sequences adjacent to the target site deletion. All inserted transgenic sequences (5778 bp) are from the plasmid pTCO113.

71. FSANZ (2017) and USDA-APHIS (2017) have previously reported that the MS11 insertion locus is on *B. napus* chromosome A03.

72. Using the *Standard Nucleotide BLAST (megablast)* online tool (Johnson et al., 2008), the nucleotide sequences of the 5' and 3' flanking sequences were compared with the standard nucleotide collection database, containing over 60 million DNA sequences. Part of the 5' flanking sequence and all of the 3' flanking sequence are homologous to part of the open reading frame and terminator sequences of a predicted endogenous gene located on chromosome A03 of *B. napus.* The T-DNA insertion site, i.e. the 40 bp target site deletion, is located 196 bases downstream of the open reading frame. Disruption of the 3' untranslated region can modify gene expression by affecting the maturation of transcribed mRNA and subsequent translation (Biłas et al., 2016; OGTR, 2019).

73. Details of the 5' and 3' flanking sequences of the T-DNA insertion and, thus, the precise location of the T-DNA insertion, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

4.3.2 Molecular characterisation and stability

74. Southern blot and PCR analysis was carried out on genomic DNA extracted from the leaves of T2 generation MS11 canola plants to determine the copy number of the inserted transgenes and check for unintended presence of plasmid backbone sequences (Anon., 2016a). Southern blot hybridisation results confirmed that the *bar*, *barnase* and *barstar* gene cassettes are present as a single complete T-DNA insertion and that vector backbone sequences are absent in MS11 canola. PCR analysis confirmed the absence in MS11 canola of a second copy of *barstar*, which is present in the pTCO113 transformation vector backbone.

75. The inheritance pattern of the introduced genes provides further evidence that the MS11 event is present as a single insertion. The frequency of the presence of the *bar*, *barnase* and *barstar* genes was measured in five generations (T3, T4, T5, BC4, BC5) of MS11 canola crossed with non-GM canola using PCR analysis (Anon., 2016c). The MS11 event is expected to segregate at a ratio of 1:1 (hemizygous to null) in the progeny of MS11 plants crossed with non-GM plants (see Figure 2). Statistical analysis confirmed that the MS11 event was inherited in a predictable manner, consistent with Mendelian principles. The three genes co-segregated as expected, being present in samples positive for MS11 and absent in samples negative for MS11.

76. The structural stability of MS11 canola was checked using Southern blot hybridisation. Following digestion with restriction enzyme *Eco*RV, a T-DNA probe hybridised with the expected fragments of genomic DNA for all samples in five generations (T2, T3, F1, BC1, BC2) of MS11 canola (Anon., 2016f).

4.3.3 Expression of the introduced proteins

77. Protein expression was measured in MS11 plants grown at three field sites in Canada and the USA in 2014 (Anon., 2016e). Enzyme-linked immunosorbent assay (ELISA) results are shown in Table 2.

78. Expression of PAT was measurable in all sampled plant tissues. Greatest concentrations were in above-ground plant tissues, i.e. whole plant and raceme, with low levels of the protein measurable in roots and grain. The highest measured concentration of PAT was 74.44 µg/g dry weight, in a treated whole plant sample harvested at 3–5 leaf growth stage.

79. Barnase could not be measured in any of the samples, as expression was below the lower limit of quantification (LLOQ). As barnase expression is controlled by a tapetum-specific promoter, expression of barnase would only be expected in samples containing tapetum tissue, e.g. whole plant at first flowering and raceme at first flowering. The fact that the barnase-barstar hybrid breeding system functions as

predicted (plants have flowers, but lack anthers), along with genomic characterisation information, indicates that the barnase protein is expressed in MS11 canola.

80. Barstar expression was predominantly measurable in roots, with expression occasionally observed in whole plants and racemes. The highest measured concentration of barstar was 1.04 µg/g dry weight, in a treated root sample harvested at stem elongation.

Table 2 Expression levels of introduced proteins in MS11 canola grown in the USA and Canada (Anon., 2016e)

Tissue	Plant growth stage (BBCH scale[a])	Treat-ment	Protein expression (µg/g dry weight ± standard deviation)		
			PAT	barnase	barstar
Whole plant	3–5 leaf (13-15)	U	22.02 ± 7.09 (4)[b]	ND [0.500][c]	ND [0.500]
		T	35.40 ± 16.22 (15)	ND [0.500]	ND [0.500]
Whole plant	Stem elongation (30-39)	U	24.68 ± 12.02 (6)	ND [1.000]	ND [0.500]
		T	21.89 ± 9.59 (14)	ND [1.000]	ND [0.500]
Whole plant	First flowering (57-65)	U	18.93 ± 9.55 (3)	ND [1.000]	ND [0.500]
		T	14.82 ± 5.01 (14)	ND [1.000]	0.21 ± 0.08 (3)
Root	Stem elongation (30-39)	U	0.17 ± 0.03 (3)	ND [2.500]	0.43 ± 0.38 (4)
		T	0.39 ± 0.19 (6)	ND [2.500]	0.50 ± 0.24 (12)
Root	First flowering (57-65)	U	0.17 ± ND (1)	ND [2.500]	0.40 ± 0.09 (3)
		T	0.37 ± 0.25 (6)	ND [2.500]	0.39 ± 0.10 (10)
Raceme	First flowering (57-65)	U	13.95 ± 1.50 (4)	ND [0.750]	ND [0.500]
		T	23.89 ± 10.73 (14)	ND [0.750]	0.68 ± 0.31 (2)
Grain	Maturity (89-99)	U	0.34 ± 0.18 (9)	ND [1.000]	ND [0.500]
		T	0.49 ± 0.18 (15)	ND [1.000]	ND [0.500]

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

[b] Numbers in parentheses indicate the number of samples above the lower limit of quantification (LLOQ). Only samples above the LLOQ were used for the calculation of the mean and standard deviation.

[c] Numbers in square brackets indicate the LLOQ in ng/mL fresh weight.

U, untreated; T, treated with glufosinate ammonium; ND, not determined as all samples were below lower limit of quantification (LLOQ); n=15 samples analysed for each row, except untreated whole plant at first flowering (n=14). Note that fewer samples > LLOQ are recorded for untreated plants, due to the inclusion of null segregants, as the presence of the MS11 event was not checked.

4.3.4 Compositional analysis of canola seed

81. The applicant provided compositional data for canola seed harvested from experimental field plots of MS11 canola, grown alongside its conventional counterpart and other non-GM reference varieties, at nine sites in Canada and the USA in 2014 (Anon., 2017b). The geographic range of the field sites was representative of commercial canola production across Canada and the USA, as it encompassed different soil types, climates and cropping systems.

82. Up to 300 g of mature canola seed was analysed from each plot. Fifty-seven analytes were measured, including proximates and fibre, amino acids, vitamins, minerals, anti-nutrients, glucosinolates and fatty acids (Anon., 2017a). Seeds of the conventional counterpart (N90-740; Entry A) were compared with seeds from plots sown to the MS11 parental canola line:

- Entry B. These plots were not treated with trait-specific herbicide, so only half of the flowering plants would have carried the MS11 event (see Figure 3a). Flowers in these plots were most likely cross-pollinated with null segregants of MS11.
- Entry C. These plots were treated with trait-specific herbicide, so all of the flowering plants would have carried the MS11 event. Flowers in these plots would have been cross-pollinated with the conventional counterpart or reference varieties[4] at the trial site.

83. The only statistically significant differences between Entry A and Entry B were for the anti-nutrients gluconapin and insoluble tannins. For gluconapin, Entry A had 2.09 $\pm$ 0.85 µmol/g DW (range 0.671–4.05 µmol/g DW) and Entry B had 2.69 $\pm$ 1.11 µmol/g DW (range 0.677–5.04 µmol/g DW). These values extend beyond the range of the non-GM reference varieties in the trial (0.723–4.83 µmol/g DW), but fall within the range of values reported for canola seed (0.10–6.84 µmol/g DW) in the AFSI Crop Composition Database (accessed November 2020). For insoluble tannins, Entry A had 0.403 $\pm$ 0.095 % DW (range 0.234–0.644 % DW) and Entry B had 0.455 $\pm$ 0.110 % DW (range 0.221–0.697 % DW). These values extend beyond the range of the non-GM reference varieties in the trial (0.043–0.604 % DW), but fall within the range of values reported for canola seed (0.07–1.32 % DW) in the AFSI Crop Composition Database (accessed November 2020).

84. Statistically significant differences were found between Entry A and Entry C for 30 analytes; however, the means were within the range of the reference varieties. The greater variation between Entry A and Entry C, compared with Entry A and Entry B, was attributed to greater genetic diversity in Entry C seeds due to cross-pollination with other canola varieties grown at the trial site.

85. FSANZ analysis assessed the GM seed to be compositionally equivalent to non-GM canola seed (FSANZ, 2017).

4.3.5 Phenotypic characterisation and environmental interaction

Phenotypic and agronomic characterisation

86. The applicant provided phenotypic and agronomic data for experimental field plots of MS11 canola, grown alongside its conventional counterpart and other non-GM reference varieties, at nine sites in Canada and the USA in 2014 (Anon., 2017b). As mentioned in Section 4.3.4, the field sites were representative of commercial canola production across Canada and the USA.

87. Measurements were taken during early season, mid-season and at crop maturity (Anon., 2016b). Continuous parameters included early and final stand count, days to flowering, days to maturity, average plant height and yield. Categorical parameters included seedling vigour, lodging, pod shattering, abiotic stress rating, disease stress rating and insect stress rating.

88. Parameters were compared between the conventional counterpart (N90-740; Entry A) and plots sown to the MS11 parental canola line:

- Entry B. These plots were not treated with trait-specific herbicide.
- Entry C. These plots were initially planted with double the seeding density of Entry A and Entry B plots, as half the plants were expected to be killed by herbicide treatment. Trait-specific herbicide was applied to Entry C plots during leaf development, at BBCH Growth Stage 12–14 (Anon., 2017b).

[4] Reference varieties were grown at each trial site, which represented the variability existing in commercial *B. napus* lines.

89. No statistically significant differences were found between the measured traits between Entry A and Entry B (Anon., 2016b). Some significant differences were observed between Entry A and Entry C; however, most mean values fell within the range and tolerance interval of the reference varieties.

- Early stand count for Entry C exceeded the reference variety values, which was expected as Entry C was sown at twice the seeding density, to allow for spraying out of null segregants during leaf development.
- Seedling vigour[5] was significantly greater for Entry C (7.53 ± 1.71) than for Entry A (6.83 ± 1.65). Differences in sowing density affect canola growth, with high sowing rates producing overly tall, weak plants (GRDC, 2015b).
- Abiotic stress rating[6] during BBCH Growth Stage 30-39 (stem elongation) was slightly, but significantly, greater for Entry C than for Entry A, i.e. Entry C plants showed slightly more signs of stress. At three other measurement time points, abiotic stress rating was not significantly different between Entry A and Entry C.

Overall, the phenotypic and agronomic characteristics of MS11 canola are considered equivalent to the conventional counterpart.

Seed germination

90. Seed germination was compared between MS11 canola and its conventional counterpart (N90-740) at warm and cold temperatures (400 and 200 seeds per lines, respectively) (Anon., 2015). No statistically significant differences were found in germination potential between the two genotypes.

Cold tolerance

91. Cold tolerance was compared between MS11 canola and its conventional counterpart (N90-740). Imbibed seeds were incubated at -10 ± 5°C for 10 days, before being incubated at temperatures conducive to germination (Anon., 2016d). Both genotypes exhibited low cold tolerance, with no statistically significant differences.

Section 5 The receiving environment

92. 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).

93. The applicant has proposed to release MS11 canola 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[7] grown in Queensland and Tasmania (ABARES, 2020). The applicant intends to use MS11 canola as a breeding and seed multiplication parental line to produce hybrid canola seed, which would be sold for commercial production. MS11 canola would not be commercialised as a standalone product. The actual locations,

[5] Seedling vigour was measured on a scale from 1 to 9, with 1 = short plants with thin leaves and 9 = tall plants with large vigorous leaves.

[6] Abiotic stress was scored on a scale from 1 to 9, with 1 = little or no stressor present, and 9 = stressor symptoms are severe; crop damage and yield loss are certain and significant.

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

number of sites and area of land used in the proposed release would depend on factors such as grower demand.

5.1 Relevant agronomic practices

94. Canola is generally sown from early April to mid-May, 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. Canola is harvested in early summer, when seeds have reached maturity and plants have dried (OGTR, 2017). Crop ripening is often hastened by windrowing (swathing) or chemical desiccation.

95. 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. 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).

96. The agronomic management of MS11 canola would differ from the management of non-GM canola and some GM canola varieties (i.e. non-glufosinate-tolerant varieties) in that glufosinate herbicide would be applied over the top of the canola crop during maintenance of the MS11 parental line and during hybrid seed production in order to destroy plants that do not carry the MS11 event. Management of volunteer canola following growing of MS11 crops would need to rely on cultivation and/or herbicide spraying using herbicides other than glufosinate.

5.2 Relevant abiotic factors

97. 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).

98. 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).

5.3 Relevant biotic factors

5.3.1 Presence of sexually compatible plants in the receiving environment

99. Gene transfer to sexually compatible plants in the receiving environment can occur via cross-pollination. Canola pollination, in general, is described in Section 3 and pollination of male sterile canola plants by sexually compatible species is discussed in Section 4.2.5.

100. 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. In 2015, the Australian canola crop

comprised of approximately 60% non-GM triazine tolerant (TT), 15% non-GM imidazolinone tolerant (IMI; Clearfield®), 20% GM glyphosate tolerant (GT; Roundup Ready®) and 5% non-herbicide tolerant canola varieties (OGTR, 2017). Stacked varieties containing two herbicide tolerance traits have also become available (TT + IMI, TT + GT, IMI + GT) (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).

101. The GM canola varieties approved for commercial cultivation in Australia are listed in Table 3. TruFlex™ canola, a newer variant of Roundup Ready® canola, became available to growers in 2019 (Shackley et al., 2019; Matthews et al., 2020). Although GM glufosinate ammonium tolerant varieties have been approved by the Regulator since 2003, the LibertyLink® trait (glufosinate ammonium tolerance) is only expected to be grown in demonstration trials in 2021 before becoming available to Australian growers in the future (BASF website, accessed November 2020).

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

102. 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).

103. *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).

104. 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).

105. 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 November 2020), *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.

106. 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; Chèvre 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 November 2020).

107. When male sterile *B. napus* is grown in close proximity to *R. raphanistrum* or *H. incana*, and flowering periods overlap, hybrid seeds are produced more readily. This situation could occur in nature if male sterile canola seeds were spilled outside of cultivation and grew in a stand of wild radish or Buchan weed. In both cases, production of hybrid seeds in the female parent (*B. napus*) is lower than if the plant had been pollinated with *B. napus* pollen (Eber et al., 1994; Darmency et al., 1998). Seed production of hybrid F1 progeny is also very low. However, if hybrid plants are able to establish and back-cross with a parent species, it is expected that fertility of the hybrid progeny could approach that of the parent species over successive generations.

108. 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).

5.3.2 Presence of related native plants in the receiving environment

109. 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).

110. 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.

5.3.3 Presence of other biotic factors

111. 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).

112. 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.

113. 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).

5.3.4 Weed resistance to glufosinate herbicides

114. There is potential for development of herbicide-resistant weeds if glufosinate is inappropriately used with MS11 canola. The repetitive use of a single herbicide, or herbicide group[8], 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.

115. 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.

116. Weeds resistant to glufosinate ammonium 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 ammonium are goosegrass (*Eleusine indica*; Malaysia), Italian ryegrass (*Lolium multiflorum*; NZ, USA), perennial ryegrass (*L. perenne*; NZ), and rigid ryegrass (*L. rigidum*, annual ryegrass[9]; Greece).

117. Stewardship guides and crop management plans (CMPs) are prepared by companies selling herbicide tolerant canola seed, e.g. Advanta Seeds (2019), GenTech Seeds Pty Ltd (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 farmer co-operators and commercial farmers with a CMP for MS11 canola and its commercial hybrid progeny. This will include the measures that were taken to manage volunteers in InVigor® hybrid canola (DIR 021/2002). The guidelines include good farm hygiene to minimise the occurrence of off-types and volunteers during production, handling, transport and storage of GM and non-GM canola.

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

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

119. 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,

[8] 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.

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

1999). 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).

120. 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.

121. 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.

Section 6 Previous authorisations

6.1 Australian authorisations of MS11 canola

122. The Regulator has previously authorised canola with the MS11 event for limited and controlled release under licences DIR 069/2006 and DIR 104. Previous assessment of MS11 canola concluded that the event poses negligible risks to human health and safety, and the environment. There were no reported adverse effects on human health or the environment from field trials grown under licences DIR 069/2006 and DIR 104.

123. A number of licences have been issued for canola with the *barnase* and *barstar* genes, in combination with *bar* and/or other introduced genes (Table 4). 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 introduced *barnase*, *barstar* and *bar* genes.

Table 4 Previous releases of canola with *barnase, barstar* and *bar* genes in combination with other GM traits in Australia

DIR licence number	Licence type	Title	Relevant genes	Additional GM agronomic traits
010/2001	L&C[a]	Small and large scale trialing of InVigor® canola (*Brassica napus*) for the Australian cropping system and seed production	*barnase, barstar, bar*	
021/2002	C[b]	Commercial release of genetically modified (InVigor® hybrid) canola	*barnase, barstar, bar*	HT[c]
032/2002	L&C	Field trial - Seed increase and field evaluation of herbicide tolerant genetically modified canola incorporating a hybrid breeding system	*barnase, barstar*	HT

DIR licence number	Licence type	Title	Relevant genes	Additional GM agronomic traits
069/2006	L&C	Limited and controlled release of GM herbicide tolerant hybrid *Brassica napus* and hybrid *Brassica juncea*	*barnase, barstar, bar*	HT
104	L&C	Limited and controlled release of canola and Indian mustard genetically modified for herbicide tolerance and/or a hybrid breeding system	*barnase, barstar, bar*	HT
108	C	Commercial release of canola genetically modified for herbicide tolerance and a hybrid breeding system (InVigor® x Roundup Ready® canola)	*barnase, barstar, bar*	HT
138	C	Commercial release of canola genetically modified for dual herbicide tolerance and a hybrid breeding system (InVigor® x TruFlex™ Roundup Ready®)	*barnase, barstar, bar*	HT

[a] L&C, limited and controlled release; [b] C, commercial release; [c] HT, herbicide tolerance

6.2 Approvals by other Australian agencies

124. 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.

125. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has approved food derived from MS11 canola as safe for human consumption (FSANZ, 2017).

126. The APVMA has regulatory responsibility for agricultural chemicals, including herbicides, in Australia. The applicant holds a registration for the use of Liberty herbicide (glufosinate ammonium) for use on InVigor® hybrid varieties of canola (APVMA PubCRIS database, accessed July 2020).

6.3 International authorisations and experience

127. A number of countries have approved MS11 canola for commercial cultivation, as well as food and feed use (Table 5).

Table 5 International approvals of MS11 canola

Country	Food - direct use or processing	Feed - direct use or processing	Cultivation - domestic or non-domestic use
Canada	2018	2018	2018
New Zealand	2017		
Philippines	2019	2019	
South Korea	2019		
Taiwan	2018		
USA	2017	2017	2017

Source: ISAAA GM approval database; accessed October 2020

128. 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 MS11 canola.

Chapter 2 Risk assessment

Section 1 Introduction

129. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 4). Risks are identified within the established risk 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.

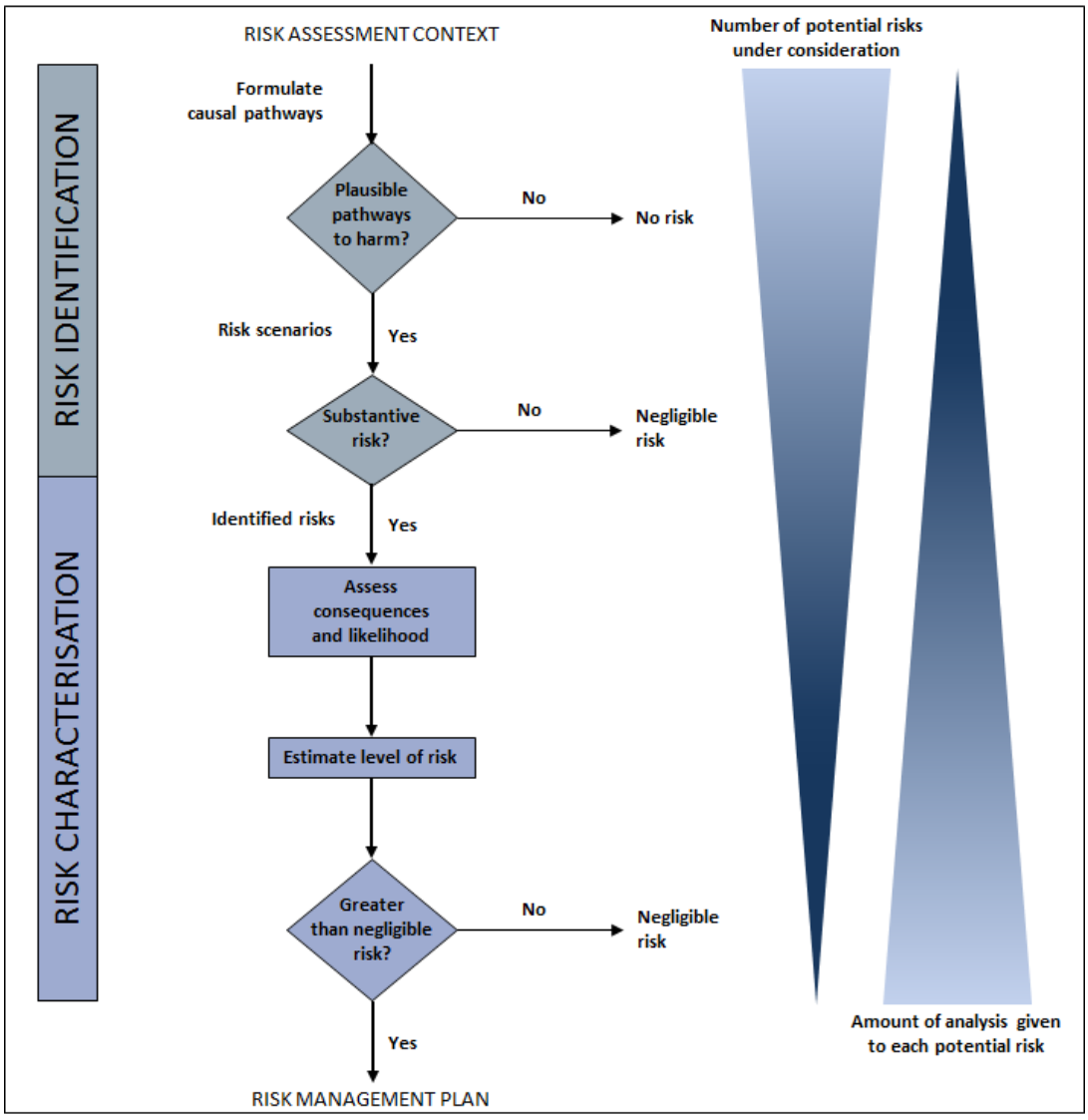

Figure 4 The risk assessment process

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

131. 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.

132. 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 4), i.e. the risk is considered no greater than negligible.

133. 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.

134. 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

135. Postulated risk scenarios are comprised of three components (Figure 5):

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.

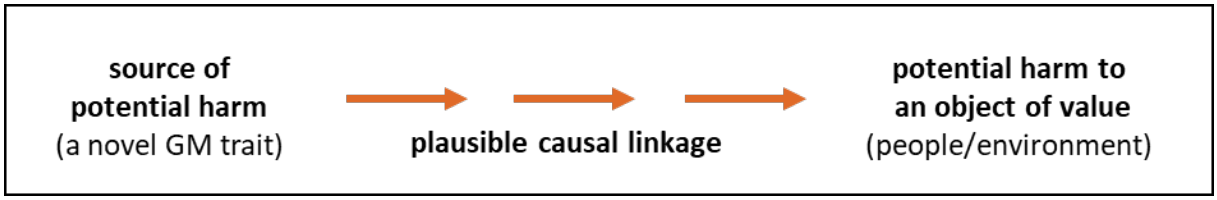

Figure 5 Components of a risk scenario

136. 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

137. 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.

138. As discussed in Chapter 1, Section 4.1.1, the GM canola proposed for release has been modified by the introduction of a gene for herbicide tolerance and two genes for a hybrid breeding system. These introduced genes and their encoded proteins are considered further as a potential source of risk.

139. The introduced genes are controlled by introduced regulatory sequences. These regulatory sequences are derived from common plants and soil bacteria (Table 1). Regulatory sequences are naturally present in plants, and the introduced elements are expected to operate in similar ways to

endogenous elements. The regulatory sequences are DNA that is not expressed as a protein, and dietary DNA has no toxicity (Society of Toxicology, 2003). 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.

140. The genetic modification has the potential to cause unintended effects in several ways including altered expression of endogenous genes by random insertion of introduced DNA in the genome, increased metabolic burden due to expression of the introduced protein, novel traits arising out of interactions with non-target proteins and secondary effects arising from altered substrate or product levels in biochemical pathways. However, these types of effects also occur spontaneously 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). With the exception of the intended male sterility trait, no biologically significant differences were found in the biochemistry, physiology or agronomic traits of MS11 canola, when compared with non-GM canola, and the introduced genes are stable (Chapter 1, Section 4.3). Therefore, unintended effects resulting from the process of genetic modification will not be considered further.

2.2 Causal pathway

141. 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.

142. 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

143. 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 4.3.5, the response of MS11 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

144. 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 5.3.1).

145. The genetic modification to the GM canola proposed for release confers tolerance to glufosinate ammonium herbicide. Four glufosinate resistant weed species have been identified overseas (Chapter 1, Section 5.3.4).

146. 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 genetically modified crop plant, the APVMA will assess the new use pattern of the chemical. Therefore, the issue of development of herbicide resistant weeds through selective pressure will not be further considered in this risk assessment. The development of herbicide tolerant weeds through gene transfer will be considered below.

2.2.3 Herbicide metabolites

147. 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 ammonium herbicide is discussed in Chapter 1, Section 4.2.3.

148. If MS11 canola is to be commercially cultivated in Australia, the potential toxicity of glufosinate ammonium and its metabolites is considered by the APVMA in its assessment of a new use pattern for registration. Ultimately, the APVMA has regulatory responsibility for the supply of agricultural chemicals, including herbicide products, in Australia. Therefore, the potential toxicity of glufosinate ammonium and its metabolites will not be further considered in this risk assessment.

2.2.4 Horizontal gene transfer

149. 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. The applicant supplied bioinformatic analysis of the likelihood of HGT, which did not provide any new evidence to suggest that HGT of the introduced DNA in MS11 canola to microorganisms could lead to harms to humans, animals or the environment (Anon., 2019). Therefore, HGT will not be assessed further.

2.2.5 Unauthorised activities

150. 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

151. 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).

152. 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

153. Five risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 6 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.

154. 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 6 Summary of risk scenarios from the proposed dealings

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced genes for tolerance to glufosinate ammonium herbicide and hybrid breeding system	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. • GM canola lines containing the introduced genes have a history of safe use. • The introduced genes and proteins are widespread in the environment.
2	Introduced gene for tolerance to glufosinate ammonium herbicide	Cultivation of GM canola expressing the introduced gene ⬇ 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 GM canola has a lower ability to spread and persist than non-GM canola. • The genetic modification only gives an advantage to the GM canola plants in managed environments where glufosinate herbicide is applied. • The GM canola can be controlled using integrated weed management.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
3	Introduced gene for tolerance to glufosinate ammonium herbicide	Cultivation of GM canola expressing the introduced gene ⬇ Dispersal of GM canola seed to nature reserves or intensive use areas ⬇ Establishment of GM canola plants in *nature reserves or intensive use areas* ⬇ 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 has a lower ability to spread and persist than non-GM 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 gene for tolerance to glufosinate ammonium herbicide	Cultivation of GM canola expressing the introduced gene ⬇ Pollination by canola with other herbicide tolerance traits ⬇ Establishment of volunteer GM canola plants with additional herbicide tolerance traits ⬇ Reduced effectiveness of weed management measures to control the volunteer GM canola plants	• Reduced establishment or yield of desirable agricultural crops, or • Increased reservoir for pests or pathogens, or • Reduced establishment of desirable native vegetation	No	• The GM canola has a lower ability to spread and persist than non-GM canola. • Multiple-herbicide tolerant hybrids can be controlled using integrated weed management.
5	Introduced gene for tolerance to glufosinate ammonium herbicide	Cultivation of GM canola expressing the introduced gene ⬇ Cross-pollination with sexually compatible species ⬇ Introgression of the introduced herbicide tolerance gene into hybrid populations ⬇ Establishment of hybrids expressing the herbicide tolerance gene ⬇ Reduced effectiveness of weed management measures to control hybrids expressing the herbicide tolerance gene	• Reduced establishment or yield of desirable agricultural crops, or • Increased reservoir for pests or pathogens, or • Reduced services from the land use	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 tolerance to glufosinate ammonium herbicide and hybrid breeding system
Causal pathway	⬇ Cultivation of GM canola 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

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

Causal pathway

156. The applicant proposes to grow the GM canola as a parental line for the production of hybrid GM canola seed, which would be sold to farmers and grown commercially in all Australian canola growing areas. Dealings with the hybrid progeny of MS11 canola, crossed with a GM fertility restoration line, would be authorised under a separate DIR licence.

157. Although MS11 canola is intended to be grown under stringent seed production conditions to maintain seed purity, this licence application requests authorisation for all dealings with the GMO, including growing MS11 canola under general canola production conditions in all canola growing areas of Australia. Thus, the GM canola could 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.

158. People involved in cultivating or processing the GM canola, or using GM canola meal as animal feed, could be exposed to plant parts or products through contact. People involved in cultivating the GM canola for its intended purpose as a parental line could be exposed to plant parts through contact.

159. MS11 canola plants do not produce pollen, so this would not be an exposure pathway.

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

161. 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, but not pollen, from the GM canola. Soil organisms, such as earthworms, would contact root exudates or decomposing plant material after harvest. Therefore, these desirable organisms would be exposed to the GM canola and plant material derived from it.

Potential harm

162. 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.

163. The *bar*, *barnase* and *barstar* genes introduced into the GM canola encode proteins that are well characterised. Based on all available information, these proteins are not known to be toxic or allergenic to humans, do not share relevant sequence homology with known toxins or allergens (Chapter 1, Section 4.2), and do not change the biochemical composition of MS11 canola seeds (Chapter 1, Section 4.3.4).

164. Although the barnase protein acts as a bacteriocin of soil bacteria when expressed by *B. amyloliquefaciens* (Chapter 1, Section 4.2.3), expression of barnase in the GM canola is at low levels and restricted to the tapetum during pollen development. Thus, only extremely low levels of barnase could come into contact with soil microorganisms.

165. FSANZ has determined that food derived from MS11 canola is as safe for human consumption as food derived from conventional (non-GM) canola varieties (Chapter 1, Section 6.2). MS11 canola has also been approved as food and/or animal feed in other countries, including Canada, New Zealand, the Philippines, South Korea, Taiwan and the USA (Chapter 1, Section 6.3).

166. There have been no reported adverse effects on human or animal health from MS11 canola or other commercial GM crops with the same introduced genes (Chapter 1, Section 6).

167. The introduced genes were isolated from common soil bacteria (Chapter 1, Section 5.4). Thus, it is expected that desirable soil organisms are regularly exposed to the introduced proteins or their degradation products.

Conclusion

168. 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 gene for tolerance to glufosinate ammonium herbicide
Causal pathway	⬇ Cultivation of GM canola expressing the introduced gene ⬇ 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

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

Causal pathway

170. The applicant proposes to grow the GM canola as a parental line for the production of hybrid GM canola seed in Australia. In order to maintain the MS11 parental canola line, it is back-crossed to a maintainer line (Figure 2). Although MS11 canola is intended to be grown under stringent seed production conditions to maintain seed purity, this licence application requests authorisation for all

dealings with the GMO, including growing MS11 canola under general canola production conditions in all canola growing areas of Australia.

171. Volunteer canola plants are likely to occur following dispersal of GM canola seeds within agricultural areas (Chapter 1, Section 3.2). 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).

172. Characterisation of the GM canola (Chapter 1, Section 4.3) showed that the phenotype of MS11 canola was equivalent to that of conventional canola, with the exception of the intended male sterility trait, i.e. MS11 canola lacks anthers. Thus, if the male sterility trait were absent, the ability of the GM canola to spread and persist is expected to be similar to that of non-GM canola.

173. Pollen dispersal is a major pathway for gene flow (Kwit et al., 2011). However, the male sterility trait limits the ability of the GM canola to spread and persist because plants carrying the MS11 event do not produce pollen. Thus, there is no potential for pollen flow from MS11 plants to other sexually compatible plants, so spread of the MS11 trait can only occur via dispersal of seeds. MS11 is a single event, meaning that the male sterility and herbicide tolerance traits are linked and are highly unlikely to assort independently during recombination.

174. The MS11 trait is hemizygous. This means that only half of the seeds produced by a plant carrying the MS11 event will carry the GM traits (Figure 2).

175. In agricultural areas, various weed management practices are used to control canola volunteers, including the use of herbicides. MS11 canola volunteers would be tolerant to the herbicide glufosinate ammonium. Thus, the effectiveness of weed management measures to control MS11 canola volunteers would be reduced if these measures included the use of glufosinate ammonium.

176. 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 ammonium 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 3.2.4). Specifically, herbicides from groups B, C, G, H, I, L and Q are available to control volunteer canola with glufosinate tolerance 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, G+M, H+I, L+Q and C+F+I) are registered for use on canola volunteers, and would effectively control canola with glufosinate tolerance. Further details of registered herbicide products are available on the APVMA PubCRIS database.

Potential harm

Reduced establishment or yield of desirable agricultural crops

177. 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.

178. MS11 canola volunteers only have a survival advantage over non-GM canola volunteers in the presence of glufosinate ammonium herbicide, and are as susceptible as non-GM canola to all herbicides other than glufosinate ammonium. The GM canola volunteers could, therefore, be controlled using integrated weed management practices (Chapter 1, Section 3.2.4), which include using a variety of other herbicides assessed and approved by the APVMA (as discussed in paragraph 176, this includes herbicides belonging to eight other mode of action groups), as well as non-

chemical management methods currently used to control non-GM canola, such as mowing, grazing or cultivation (Australian Oilseeds Federation, 2019).

Increased reservoir for pests or pathogens

179. Canola crops are susceptible to a range of pests and diseases (Chapter 1, Section 5.3.3). 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).

180. Characterisation of the GM canola did not reveal any significant differences between the GM canola and conventional canola for disease stress or insect stress ratings (Chapter 1, Section 4.3.5). Effective control of canola volunteers (both GM and non-GM) reduces the potential for volunteers to act as reservoirs for pests and diseases.

Conclusion

181. Risk scenario 2 is not identified as a substantive risk because the GM canola has a lower ability to spread and persist than non-GM canola, the genetic modification only gives an advantage to the GM canola plants in managed environments where glufosinate ammonium herbicide is applied, and because the GM canola can be controlled by integrated weed management, such as using other herbicides and physical methods. 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 gene for tolerance to glufosinate ammonium herbicide
Causal pathway	⬇ Cultivation of GM canola expressing the introduced gene ⬇ Dispersal of GM canola seed to nature reserves or intensive use areas ⬇ Establishment of GM canola plants in *nature reserves or intensive use areas* ⬇ 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

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

Causal pathway

183. The applicant proposes to grow the GM canola as a parental line for the production of hybrid GM canola seed in Australia. As this licence application requests authorisation for all dealings with the GMO, MS11 canola could also be grown under general canola production conditions in all canola growing areas of Australia. Seeds could be dispersed before or after harvest of a MS11 crop.

184. 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. 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. However, surveys

of roadside canola typically only found feral canola plants within five metres of the edge of the road (Agrisearch, 2001). As discussed in Chapter 1, Section 3.2.3, 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.

185. 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.

186. 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).

187. The GM canola proposed for release is similar to non-GM canola with respect to most of the intrinsic characteristics contributing to spread and persistence, such as germination, seedling vigour, seed production and pod shattering (Chapter 1, Section 4.3.5). However, as discussed in Risk scenario 2, the male sterility trait reduces the ability of the GM canola to spread and persist, compared with non-GM canola.

188. If MS11 canola seed is dispersed into nature reserves or intensive use areas, the seeds could germinate and establish a population of GM plants. Half of the seeds produced by MS11 canola plants would be expected to carry the MS11 event. As the genetic modification is not expected to alter the tolerance of GM plants to biotic or abiotic stresses that normally restrict the geographic range and persistence of canola, these feral GM canola plants are not expected to be more persistent than non-GM canola.

189. The effectiveness of weed management measures to control feral GM canola would be reduced if these measures included the use of glufosinate ammonium.

Potential harm

Reduced establishment of desirable native vegetation

190. If the GM canola expressing the introduced gene for glufosinate ammonium 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.

191. In nature reserves where glufosinate ammonium is not used for weed control, the GM canola would not be expected to have any survival advantage over non-GM canola. Canola is not a significant weed in natural undisturbed habitats in Australia (Chapter 1, Section 3.2.3).

Reduced services from the land use

192. 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.

193. The glufosinate ammonium tolerance trait could affect a GM plant's tolerance to weed management practices in areas where this herbicide is used. The main herbicide used for roadside weed management in Australia is glyphosate (Storrie, 2018), which would control this GM canola. It is possible that glufosinate ammonium could be more widely used to control roadside weeds in future. However, as discussed in Risk scenario 2, canola can be controlled in agricultural settings using integrated weed management practices, including other herbicides and non-chemical methods (Chapter 1, Section 3.2.4). Similarly, canola can be controlled along roadsides using other herbicides or by slashing.

Conclusion

194. Risk scenario 3 is not identified as a substantive risk because the GM canola has a lower ability to spread and persist than non-GM canola, the GM canola is susceptible to the biotic or abiotic stresses that normally restrict the geographic range and persistence of canola, and the GM canola can be controlled by integrated weed management, such as using other herbicides and physical methods. 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 gene for tolerance to glufosinate ammonium herbicide
Causal pathway	⬇ Cultivation of GM canola expressing the introduced gene ⬇ Pollination by canola with other herbicide tolerance traits ⬇ Establishment of volunteer GM canola plants with additional herbicide tolerance traits ⬇ Reduced effectiveness of weed management measures to control the volunteer GM canola plants ⬇
Potential harm	Reduced establishment or yield of desirable agricultural crops OR Increased reservoir for pests or pathogens OR Reduced establishment of desirable native vegetation

Risk source

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

Causal pathway

196. The applicant proposes to grow the GM canola as a parental line for the production of hybrid GM canola seed in Australia. As this licence application requests authorisation for all dealings with the GMO, MS11 canola could also be grown under general canola production conditions in all canola growing areas of Australia.

197. MS11 canola could cross-breed with other herbicide tolerant canola plants either intentionally or unintentionally. The licence applicant would not be authorised to intentionally breed MS11 canola with GM canola carrying another herbicide tolerance trait, as the GM hybrid would require a separate licence from the Regulator. However, the applicant could intentionally breed MS11 canola with non-GM canola carrying another herbicide tolerance trait, in order to produce and market dual herbicide tolerant GM canola seeds. It is unlikely that the applicant would intentionally breed MS11 canola to produce canola with more than two herbicide tolerance traits, as there are currently no such canola seeds in the market (Chapter 1, Section 5.3.1), suggesting a lack of demand. However, dual herbicide tolerant MS11 canola could breed with other herbicide tolerant canola unintentionally.

198. A herbicide tolerance trait could potentially be transferred unintentionally to MS11 canola by pollen flow from other canola, including other herbicide tolerant non-GM and GM canola plants. MS11 canola is more likely to outcross than non-GM canola, due to the male sterility trait. Canola is predominantly self-pollinating (Chapter 1, Section 3). As MS11 canola is male sterile, cross-fertilisation with pollen from sexually compatible plants is required in order to produce seeds. Cross-

fertilisation, mediated by wind or insects, usually occurs over short distances (less than 10 m); however, long-distance pollen flow is possible (Chapter 1, Section 4.2.5).

199. If MS11 canola is grown for maintenance of the MS11 parental canola line, it would be grown in proximity to a non-GM maintainer line for back-crossing (Figure 2). Likewise, if MS11 canola is being grown for the purpose of hybrid sowing seed production, it would be grown in proximity to a fertility restoration (RF) line. In both cases, the grower would follow seed production protocols and ensure suitable isolation distances from undesirable sexually compatible species in order to ensure genetic purity of the seed.

200. If canola seeds that were progeny of MS11 canola were grown as a general commercial crop, it is expected that half of the plants would lack the MS11 event and would be male fertile. Most pollination of the MS11 plants would be by adjacent null-segregant plants. A small amount of outcrossing could occur between MS11 canola and nearby canola crops with other herbicide tolerance traits.

201. In addition to glufosinate ammonium tolerance (LibertyLink®), there are currently three herbicide tolerance traits in Australian canola varieties:

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

Canola with glufosinate ammonium tolerance has been approved for commercial cultivation in Australia since 2003; however, the LibertyLink® trait has not yet become available to farmers (Chapter 1, Section 5.3.1). The GM canola varieties with glufosinate ammonium tolerance that were authorised for commercial release under DIR 021/2002, DIR 108 and DIR 138 have only been planted in field trial settings and small, non-commercial scales to date.

202. If MS11 canola were to cross with TT, IMI and GT canola, this could result in a canola with tolerance to four herbicides. This has been theoretically possible since the approval of InVigor® canola and Roundup Ready® canola in 2003. Approval of MS11 canola for commercial release would not add a new trait to the combinations of herbicide tolerance possible in canola volunteers.

203. Hybrid seed with additional herbicide tolerance traits could disperse within agricultural areas, to intensive use areas or to nature reserves by the same mechanisms described in Risk scenarios 2 and 3. Volunteer or feral progeny of MS11 canola plants could germinate and grow in these areas.

204. As discussed in Risk scenario 2, the herbicide tolerance gene is linked to the male sterility gene. This reduces the ability of MS11 canola to spread and persist; however, the introduced genes are not expected to alter the tolerance of GM plants to biotic or abiotic stresses. Therefore, hybrids with additional herbicide tolerance traits would likely be less invasive and persistent than equivalent hybrids that do not possess the MS11 male sterility trait.

205. If hybrid progeny with multiple herbicide tolerance traits were to establish, the effectiveness of existing weed management measures to control volunteer or feral canola could be compromised. Depending on the herbicide tolerance traits, the following mode of action groups may not be available to control canola volunteers: Group B (imidazolinone), Group C (triazine) and Group M (glyphosate), along with Group N (glufosinate ammonium).

Potential harm

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

207. If volunteer canola were able to establish and persist in intensive use areas or nature reserves, this could affect the growth of native vegetation or reduce services from the land uses, as described in Risk scenario 3. In the absence of herbicide application, multiple-herbicide resistant canola plants would not be expected to have a survival advantage over non-GM canola. Multiple-herbicide resistant canola could be controlled with alternative weed management in nature reserves and intensive use areas, as discussed in Risk scenario 3.

Conclusion

208. Risk scenario 4 is not identified as a substantive risk because the GM canola has a lower ability to spread and persist than non-GM canola and multiple-herbicide tolerant hybrids can be controlled by integrated weed management, such as using other herbicides and physical methods. 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 gene for tolerance to glufosinate ammonium herbicide
Causal pathway	⬇ Cultivation of GM canola expressing the introduced gene ⬇ Cross-pollination with sexually compatible species ⬇ Introgression of the introduced herbicide tolerance gene into weed populations ⬇ 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 OR Increased reservoir for pests or pathogens OR Reduced services from the land use

Risk source

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

Causal pathway

210. The applicant proposes to grow the GM canola as a parental line for the production of hybrid GM canola seed in Australia. As this licence application requests authorisation for all dealings with the GMO, MS11 canola could also be grown under general canola production conditions in all canola growing areas of Australia.

211. Cultivation of the GM canola could bring it into proximity to other Brassica crop species, such as vegetables, forage crops and Indian mustard, as well as related weed species. As discussed in Risk scenario 4, MS11 canola is more likely to be cross-fertilised with pollen from other sexually compatible plants than non-GM canola, as it cannot self-pollinate. However, the likelihood of outcrossing with sexually compatible non-canola species is low when MS11 canola is grown in the field, as male fertile canola would typically be grown in close proximity for the purpose of pollination.

212. If MS11 canola seeds were dispersed into field margins or other agricultural areas, into intensive use areas, or into nature reserves, this could increase the likelihood of pollen flow from sexually compatible species to MS11 canola, if flowering was synchronous.

Interactions with Brassica crop species

213. Pollen flow from Brassica crop species other than canola to MS11 canola 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 5.3.1). Brassica forage crops usually do not reach flowering. *Brassica juncea* (Indian mustard) crops, which are grown as oilseeds or for condiment mustard, could plausibly cross-pollinate with the GM canola. Cross-pollination could also occur with Brassica volunteers.

214. Hybridisation between MS11 canola and other Brassica crop species could occur if the GM canola is released (Chapter 1, Section 5.3.1). However, the frequency of interspecies crossing would be lower than the frequency of crossing between MS11 canola and other canola plants, because there is greater sexual compatibility between *B. napus* plants than between *B. napus* and other species. In Risk scenario 4, it was considered that unintended hybridisation between MS11 canola and other canola would occur at low levels, so hybridisation between MS11 canola and other Brassica crop species is likely to occur at very low levels.

Interactions with Brassicaceae weeds

215. 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.*

216. 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 5.3.1). When canola is pollinated by a weed species, fewer hybrid seeds are produced than if it had been pollinated by another canola plant. Any hybrid progeny also produce fewer seeds; however, fertility can approach that of the parent species if the progeny are allowed to establish and back-cross over successive generations.

217. Hybridisation between *B. napus* and other Brassicaceae weeds under open-pollination field conditions is highly unlikely (OGTR, 2017).

218. If hybridisation were to occur between MS11 canola and sexually compatible weed species, the introduced gene sequences on chromosome A03 would need to introgress into the hybrid genome, in order to be inherited by subsequent generations. Introgression of regions of the *B. napus* A03 chromosome into *R. raphanistrum* has been observed, but at a lower rate than introgression of regions of other *B. napus* chromosomes (Chapter 1, Section 5.3.1).

Interactions with native Brassicaceae

219. Hybridisation between *B. napus* and Australian native Brassicaceae is not plausible under natural conditions (Chapter 1, Section 5.3.2). Australian native Brassicaceae are more distantly related to *B. napus* than the weeds discussed in the previous section (*H. incana*, *R. raphanistrum* and *S. arvensis*), which belong to the tribe Brassiceae. Naturally occurring hybrids between *B. napus* and weeds in the tribe Brassiceae are rare, so it is almost certainly impossible for *B. napus* to hybridise with members of different tribes.

Establishment of hybrids expressing the herbicide tolerance gene

220. If the herbicide tolerance gene was present in plants that are GM hybrids between MS11 canola and other Brassica crop species, these plants could establish as volunteers in agricultural areas or as feral plants in nature reserves or intensive use areas.

221. In the highly unlikely event that the herbicide tolerance gene was introgressed into a population of wild radish, Buchan weed or charlock, and this population retained the vigour of the recurrent weedy parent, these plants could establish as weeds.

222. As discussed in Risk scenario 2, the herbicide tolerance gene is linked to the male sterility gene. This reduces the ability of MS11 canola to spread and persist; however, the introduced genes are not expected to alter the tolerance of GM plants to biotic or abiotic stresses. Therefore, GM hybrids between MS11 canola and sexually compatible species would likely be less invasive and persistent than equivalent hybrids between non-GM canola and sexually compatible species.

223. The GM hybrids would not be controlled by the application of glufosinate ammonium herbicide.

Potential harm

224. Both volunteer canola and other Brassica crop species are weeds of agricultural production systems (Groves et al., 2003). Any hybrids between MS11 canola and other Brassica species could also potentially become volunteers. If left uncontrolled, GM hybrid volunteers could reduce the establishment or yield of desired crops, through direct competition or by providing a reservoir for pests or pathogens.

225. 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 5.3.1). If the GM herbicide tolerance trait were introgressed into a population of one of these weeds, it would increase the difficulty of weed management when glufosinate ammonium herbicide is used. These GM weeds could impact the agricultural environment by reducing the establishment or yield of desired crops.

226. Wild radish and Buchan weed are also common roadside weeds (Chapter 1, Section 5.3.1). If the GM herbicide tolerance trait introgressed into these weeds, the GM weeds could reduce services from the land use if glufosinate ammonium herbicide was applied to control weeds in these areas.

227. 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.

Conclusion

228. Risk scenario 5 is not identified as a substantive risk because 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, 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

229. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis[10]. 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.

230. 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.

231. MS11 canola has been approved by the Regulator for limited and controlled release (field trial) under licences DIR 069/2006 and DIR 104. The RARMPs for DIR 069/2006 and DIR 104 identified additional information that may be required for a large scale or commercial release of MS11 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 4.3, and discussed in relevant sections in Chapter 1 and in risk scenarios.

232. Uncertainty can arise from a lack of experience with the GMO. MS11 canola has only been grown in Australia under limited and controlled (field trial) conditions. However, the level of uncertainty is considered to be low, given that the MS11 canola and earlier generation GM canola containing the *bar, 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.

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

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

[10] 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.

Section 4 Risk evaluation

235. 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.

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

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

237. 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 6.

238. 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

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

240. 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.

241. 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.

242. 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

243. 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 MS11 canola. These risk scenarios were considered in the context of the scale of the proposed release and the receiving environment. The risk evaluation concluded that no containment measures are required to treat these negligible risks.

Section 3 General risk management

244. 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

245. 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.

246. 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.

247. 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

248. 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.

249. As part of the licence application package, BASF provided a real-time PCR method for the identification and quantification of the relative content of the MS11 event DNA in a *B. napus* DNA test sample (Bayer CropScience, 2016).

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

250. Any person, including the licence holder, can conduct any permitted dealing with the GMO.

3.4 Reporting requirements

251. 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.

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

253. There are also provisions that 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

254. 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.

255. 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

256. 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.

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

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

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

4.1 Adverse effects reporting system

258. 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

259. 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.

260. 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.

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

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

263. 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

264. 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

265. The risk assessment concludes that the proposed commercial release of GM canola (MS11) poses negligible risks to the health and safety of people or the environment as a result of gene technology.

266. 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 (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.

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.

Anon. (2008). Full DNA sequence of event insert and integration site of *Brassica napus* transformation event MS11. Document number M-304805-01-1. (Bayer CropScience, unpublished).

Anon. (2015). MS11 *Brassica napus* - Seed germination potential, 2015. Final report. Document number M-528906-01-1. (Bayer CropScience, unpublished).

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

Anon. (2016b). MS11 *B. napus* - Agronomic assessment of MS11 *B. napus g*rown in Canada and the USA during 2014. Document number M-549078-01-1. (Bayer CropScience, unpublished).

Anon. (2016c). MS11 *Brassica napus* - inheritance of the insert over generations. Document number M-545765-01-2. (Bayer CropScience, unpublished).

Anon. (2016d). MS11 *Brassica napus* - Seed cold tolerance, 2015. Final report. Document number M-547811-01-1. (Bayer CropScience, unpublished).

Anon. (2016e). MS11 *Brassica napus* – Summary of protein expression analyses of field samples grown in Canada and the USA during 2014. Document number M-549123-01-1. (Bayer, unpublished).

Anon. (2016f). Structural stability analysis of *Brassica napus* MS11. Document number M-547544-01-1. (Bayer, unpublished).

Anon. (2017a). MS11 *B. napus* – composition analysis of field samples grown in Canada and the USA during 2014. Document number M-549080-02-1. (Bayer, unpublished).

Anon. (2017b). MS11 x RF3 and MS11 *B. napus* - Field Production in Canada and the USA during 2014. Document number M-549076-02-1. (Bayer, unpublished).

Anon. (2019). Identity of the MS11 *Brassica napus* insert sequences to known microbial DNA sequences and assessment of the potential for Horizontal Gene Transfer. Document number 19-RSOS0054-EU. (BASF, unpublished).

ANZFA (2001). 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.

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

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).

Bayer CropScience (2016). Real-Time PCR method for event-specific quantification of *Brassica napus* GM event MS11. Document number M-558702-02-1. (Bayer CropScience, unpublished).

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.

Biłas, R., Szafran, K., Hnatuszko-Konka, K., and Kononowicz, A.K. (2016). *Cis*-regulatory elements used to control gene expression in plants. Plant Cell, Tissue and Organ Culture *127*, 269-287.

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).

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.

Chèvre, 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).

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.

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.

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

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.

Eber, F., Chèvre, A.-M., Baranger, A., Vallée, P., Tanguy, X., and Renard, M. (1994). Spontaneous hybridization between a male-sterile oilseed rape and two weeds. Theoretical and Applied Genetics *88*, 362-368.

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).

EFSA GMO Panel, Naegeli, H., Bresson, J.-L., Dalmay, T., Dewhurst, I.C., Epstein, M.M., Firbank, L.G*., et al.* (2020). Assessment of genetically modified oilseed rape MS11 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-BE-2016-138). European Food Safety Authority (EFSA) Journal *18*, e06112.

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

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).

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

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

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

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

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).

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).

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.

Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S., and Madden, T.L. (2008). NCBI BLAST: a better web interface. Nucleic Acids Research *36*, W5-9.

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.

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

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 thaliana*. Plant Molecular Biology *11*, 745-759.

Kwit, C., Moon, H.S., Warwick, S.I., and Stewart, C.N. (2011). Transgene introgression in crop relatives: molecular evidence and mitigation strategies. Trends in Biotechnology *29*, 284-293.

Lankinen, Å., Lindström, S.A.M., and D'Hertefeldt, T. (2018). Variable pollen viability and effects of pollen load size on components of seed set in cultivars and feral populations of oilseed rape. PLoS ONE *13*, e0204407 (0204401-0204415).

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.

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.

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.

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

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

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

OECD (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 (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 (2019). Risk assessment reference: Regulatory sequences in GM plants. (Canberra, Australia: Office of the Gene Technology Regulator).

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.

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).

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.

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).

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 (2017). Petition number 16-235-01p (Extension of 98-278-01p): Updated petition. (United States Department of Agriculture Animal and Plant Health Inspection Service).

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., 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.

Zhang, C.J., Yook, M.J., Park, H.R., Lim, S.H., Kim, J.W., Song, J.S., Nah, G*., et al.* (2018). Evaluation of maximum potential gene flow from herbicide resistant *Brassica napus* to its male sterile relatives under open and wind pollination conditions. Science of the Total Environment *634*, 821-830.

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[11] 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	The committee agrees that the following should be considered in the RARMP: • The potential for the GM canola to be harmful to people through toxicity or allergenicity. • The potential for the GM canola to be harmful to other organisms through toxicity. • The potential for the introduced traits to increase the weediness of the GM canola, leading to harm to the environment. • The potential for harm to result from gene flow to other canola. The potential for commercial release to result in changes to agricultural practices that may have an environmental impact.	The potential for the GM canola to be toxic or allergenic to people or toxic to other organisms is addressed in Chapter 2, Section 2.4.1 (Risk scenario 1). The potential for increased weediness of the GM canola is addressed in Chapter 2, Sections 2.4.2 and 2.4.3 (Risk scenarios 2 and 3). The potential for crossing between the GM canola and other canola is addressed in Chapter 2, Section 2.4.4 (Risk scenario 4). Chapter 1, Section 5.1 discusses that agricultural practices for the GM canola only differ from normal seed production protocols due to application of glufosinate ammonium herbicide. Environmental impacts of glufosinate ammonium are regulated by the Australian Pesticides and Veterinary Medicines Authority.
	The committee recommends that the potential for harm to result from gene flow to other sexually compatible species including canola should be considered in the RARMP.	The potential for harm to result from gene flow to other sexually compatible species is discussed in Chapter 2, Sections 2.4.4 and 2.4.5 (Risk scenarios 4 and 5).
2	Council has no comment in regards to the commercial release of GM cotton.	Noted.
3	As Council does not have a specialist scientific expert to make an assessment, no comment will be provided.	Noted.
4	Council have no issues.	Noted.
5	Although not being an expert in the field of genetically modified cropping, one concern I believe that Council may be interested in making mention of, would be the potential risk of herbicide tolerant strain of canola becoming an uncontrollable pest that may effect our already exhausted resources. As I am sure you are aware	The potential for weediness of the GM canola, due to reduced effectiveness of herbicides, in agricultural areas, intensive use areas and nature reserves is discussed in Chapter 2, Sections 2.4.2 to 2.4.4 (Risk scenarios 2 to 4).

[11] 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
	Regional Councils often have limited Weed Control Officers to mitigate the existing issues, for example, the control of Green Cestrum on stock routes and rural properties.	
6	Shire lies within an area which supports about 8,000 taxa of vascular plants, representing two thirds of the estimated plant taxa in WA and over 80% of the plant taxa are unique. Council previously considered the issue of GM crops and foods, and passed a motion in 2009 with the following key points: 1. Shire does not have jurisdiction over the growth, transport or sale of either GM crops or GM food; 2. Council lacks sufficient scientific knowledge to reach an overall conclusion on whether genetic modification of crops is harmful or not to human health and the environment; 3. Negative perceptions of GM crops and GM food exist in the residents and some market destinations have the potential to harm the marketing of organics and other local produce, if the region was to become associated with GM crops; Council therefore does not support the use of GM crops in the shire.	Noted. 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 that are unrelated to human health and safety and the environment.
	Community concerned that there could be a potential contamination of local biodiversity with insect-borne GM pollen or organisms. This could have negative environmental impacts on the shire, with a risk of spread throughout the environment, resulting in the modification in the indigenous flora.	The potential for negative impacts on biodiversity (desirable organisms and native vegetation) due to the GMO is discussed in Chapter 2, Sections 2.4.1 and 2.4.3 (Risk scenarios 1 and 3). The potential of gene transfer from the GM canola to indigenous flora is discussed in Chapter 2, Section 2.4.5 (Risk scenario 5).
	As such, the Shire does not support the proposed commercial release of GM Canola (DIR175).	Noted.
7	Relevant experts within the Government examined the application summary. At this stage of the application process, the Government does not have specific advice on risks to the health and safety of people and the environment to be considered in the development of the consultation RARMP. I note that there is an opportunity to comment on the draft RARMP which is anticipated to be available in December 2020. The Government would welcome this opportunity to comment.	Noted.
8	The members considered the application for DIR 175-Commercial release of canola (*Brassica napus*) genetically modified for herbicide	Noted.

Submission	Summary of issues raised	Comment
	tolerance and a hybrid breeding system (MS11) from BASF Australia Ltd (BASF) out of session. At this stage the members' do not have specific comments on the application and they look forward to receiving the Risk Assessment and Risk Management Plan (RARMP) for this application in the future.	
9	While the Council is broadly supportive of application DIR 175, it has been raised that based the information provided in this application and that available on the OGTR website, does not provide sufficient information to draw a reasoned conclusion on whether this GM canola is safe or not. The Council will wait until the final submission is out for comment before it is safe to draw a conclusion.	Noted.
10	The Department believes that the proposed release of GM canola will pose a negligible risk to the environment as canola is not classified as a weedy species in natural environments, canola has a poor competitive ability in natural environments, and the GM traits are unlikely to increase weediness potential of the GM plants or weedy relatives, if gene flow to weedy species occurred.	Noted.
	Previous RARMPs on very similar GM canola lines and GM canola with different traits will be highly relevant to the preparation of the current RARMP.	As discussed in Chapter 2, Section 1, risk scenarios in previous RARMPs prepared for similar GMOs are considered when postulating risk scenarios.
	The Department recommends that the RARMP covers all potential pathways to harm, including seed-mediated gene transfer and the factors that impact seed dispersal or restrict spread and persistence in natural ecosystems. The potential for the genetic modification to increase spread and persistence due to altered factors such as tolerance to abiotic stresses should also be considered in the preparation of the RARMP.	
	Seed dispersal as a pathway to harm While it is recognised that pollen or seed dispersal are unlikely to lead to an environmental harm, both should be assessed fully in the RARMP as potential causal pathways for gene flow and potential pathways to harm. The RARMP should also discuss any factors that may limit seed dispersal by wind, water, birds and animals. ***Seed dispersal via animals and birds*** Animals and birds can eat canola seed and excrete viable seeds. Previous RARMPs have stated that because no control measures are used for birds in Australian canola, no controls for birds are warranted. However, several bird	Seed dispersal is considered as a pathway to harm in Chapter 2, Sections 2.4.2 and 2.4.3 (Risk scenarios 2 and 3). Seed dispersal, in combination with other causal pathways, is also considered in Chapter 2, Sections 2.4.4 and 2.4.5 (Risk scenarios 4 and 5). As this application is for commercial release of MS11 canola in all canola growing regions of Australia, the applicant is proposing to grow the GM canola using standard agricultural practices for canola. The applicant is not proposing any measures to manage seed dispersal, so

Submission	Summary of issues raised	Comment
	species feed on canola and the reason that no controls are used is likely to be because there are no effective controls for pest birds in broad acre crops. Additional information on studies that may support the low likelihood of viable seed dispersal by animals or birds should be included in the RARMP. Also, any information that may support the low likelihood of survival or persistence of dispersed seed should be included in the RARMP. Although endozoochory likely occurs at very low levels, it should be discussed considering the potentially large number of seeds produced and the uncertainty regarding endozoochory in animals (e.g. mice) and certain birds. ***Seed dispersal by wind*** Wind is a vector for dispersal of canola seed due to a number of factors such as high seed numbers, small seed size and seed pod shattering. The application document (p.62) for this GMO refers to wind as not being very effective at seed dispersal. However, research on GM canola in Australia demonstrated dispersal and persistence of over 300 windrowed plants into native bushland by a windstorm (Busi and Powles, 2016; DOI: 10.1016/j.agee.2015.12.028).	management of seed dispersal is not discussed in the RARMP. Pollen dispersal is not considered as a pathway to harm, as the GM canola does not produce pollen (see Chapter 1, Section 4.2.4).
	Impact of the genetic modification on seed dispersal and survival Abiotic and biotic stresses normally restrict survival, spread and persistence of canola. Research on GM canola in Australia demonstrated seed dispersal into a natural environment. However, within 3 years the population was extinct and this was primarily attributed to proliferation of blackleg fungal disease and a less than 50% average rainfall in 2010 (Busi and Powles, 2016). Other factors that influenced survival included seed predation by rabbits, birds and ants. Possible impacts that the genetic modification may have on seed dispersal (e.g. increased pod shattering) or on survival of seed (e.g. abiotic stress tolerance) should be discussed. The applicant provided a report that details studies comparing non-GM canola and GM canola from 10 field trials carried out in Canada and the US in 2014. The data shows that several factors, such as seedling vigour, pod shattering and tolerance to abiotic stresses (e.g. drought), increased in the GM canola plants. There was no increase observed in biotic (insect and disease) stress	Chapter 1, Section 4.3, discusses the phenotypic, agronomic and seed composition characteristics of MS11 canola in comparison to non-GM canola.

Submission	Summary of issues raised	Comment
	tolerances. However, the document states no biologically significant differences were observed. Considering that there are differences observed for abiotic stresses (Attachment 2; Table 29, p72) when comparing this GM canola with its non-GM counterpart, a full assessment of potential increased abiotic tolerance and impact on survival should be included in the RARMP.	These differences in abiotic stress ratings are discussed in Chapter 1, Section 4.3.5. Tolerance of the GM canola to abiotic stresses is further discussed in Chapter 2, Section 2.2.1.
11	The committee considered the application and members provided the following comments: • No concerns. GM canola has been around for nearly 20 years in Australia. The newer genes added to this one are related to hybrid generation. • No problems. It's been approved before and is a standard extension.	Noted. Canola with the MS11 event has been previously approved for limited and controlled (field trial) release by the Regulator. This application is for commercial release of this GM canola in all canola growing regions of Australia.

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	Shire lies within an area which supports about 8,000 taxa of vascular plants, representing two thirds of the estimated plant taxa in WA and over 80% of the plant taxa are unique. Previously considered the issue of GM crops and foods, and passed a motion in 2009 with the following key points: 1. Shire does not have jurisdiction over the growth, transport or sale of either GM crops or GM food; 2. Lacks sufficient scientific knowledge to reach an overall conclusion on whether genetic modification of crops is harmful or not to human health and the environment; 3. Negative perceptions of GM crops and GM food exist in the residents and some market destinations have the potential to harm the marketing of organics and other local produce, if the region was to become associated with GM crops; Does not support the use of GM crops in the shire.	Noted. 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 that are unrelated to human health and safety and the environment.
	Community concerned that there could be a potential contamination of local biodiversity with insect-borne GM pollen or organisms. This could have negative environmental impacts on the shire, with a risk of spread throughout the environment, resulting in the modification in the indigenous flora.	The potential for negative impacts on biodiversity (desirable organisms and native vegetation) due to the GMO is considered in the RARMP (Chapter 1, Section 5.3 and Chapter 2, Sections 2.4.1, 2.4.3 and 2.4.4). The potential of gene transfer from the GM canola to indigenous flora is considered in the RARMP (Chapter 1, Section 5.3.2 and Chapter 2, Section 2.4.5). The RARMP concluded that there is negligible risk associated with these risk scenarios.
	Does not support the proposed commercial release of GM Canola (DIR175).	Noted.
2	Does not have a specialist scientific expert to make an assessment, no comment will be provided.	Noted.
3	Agrees with the overall conclusion of the RARMP.	Noted.

Submission	Summary of issues raised	Comment
4	While broadly supportive of the RARMP for DIR 175, it is important to note that as per the ACT Gene Technology (GM Crop Moratorium) Act 2004 there are currently 2 Moratorium Orders that prohibit the use, release and propagation of the following introduced genes of genetically modified Canola in the ACT: - *Streptomyces hygroscopicus* - *Bacillus amyloliquefaciens* Therefore, if a licence is granted for DIR 175, then the use of these genetically modified genes would remain prohibited within the ACT.	Noted.
5	Agrees that the proposed release of GM canola will pose a negligible risk to the environment as canola is not classified as a significant weedy species in Australian natural environments, GM canola is not competitive in natural environments and the GM trait of herbicide tolerance (HT) is unlikely to increase weediness potential of the GM plants or of weedy relatives, if gene flow to weedy species were to occur. Recommends that the RARMP include additional information in support of this conclusion, in particular regarding potential altered abiotic stress tolerance and potential seed dispersal to natural ecosystems. **The RARMP should include data and discussion to support the conclusions of risk scenario 3 that no increase in abiotic stress is expected.**	Noted.
	While the RARMP states 'the applicant has addressed the uncertainty around altered abiotic and biotic stress tolerances', we recommend RS3 include additional information from the applicant on field trials conducted in Canada and the US in 2014. Data from these studies indicates that tolerance to abiotic stress (e.g. drought) increased significantly in GM canola plants compared to non-GM canola at several individual sites, with no increase observed in biotic (insect and disease) stress tolerances. RS3 should provide information to support the conclusion that no consistent or 'biologically meaningful' difference was observed when combined site analyses were performed.	Abiotic stress results from field trials conducted in Canada and the US in 2014 are discussed in Chapter 1, Section 4.3.5. Abiotic stress rating was scored at four growth stages. At one growth stage, Entry C (treated MS11) had a slightly, but significantly, higher abiotic stress rating than Entry A (conventional counterpart). This means that some stress symptoms were present in the GM canola, while less or no stress was present in the conventional counterpart. In other words, tolerance to abiotic stress *decreased* significantly in GM canola plants compared with non-GM canola at this growth stage. As no evidence was presented of the GM canola being more tolerant to abiotic stress than its non-GM counterpart and as the introduced traits are not expected to alter tolerance to abiotic stress, tolerance to abiotic factors was not further assessed in the risk scenarios (see Chapter 2, Section 2.2.1).

Submission	Summary of issues raised	Comment
	The transferability of field trial data from other countries should also be discussed. While field trial data and environmental risk assessments from most highly domesticated crops will be transferable, 'for host crops that have relatively high weediness potential or wild relatives, such as canola and soybean, further considerations are required to decide transportability of confined field trial data' . Transportability also depends on the similarity of agro-climatic zones, however 'strict similarity of environmental conditions does not seem to be necessary for highly domesticated crops such as cotton and corn to detect changes related to weediness'. RS 3 should discuss the similarity of environments for the acceptance of US and Canadian data on GM canola.	Canola is cultivated across a range of agro-climatic zones. Field trial data was collected from nine sites in Canada and the USA; these sites were located in cold or arid climate zones. In Australia, canola is grown in temperate and arid climate zones. The licence for DIR 175 allows BASF to breed the GM canola, in order to introduce the MS11 traits into canola lines suitable for Australian environments. No evidence of unexpected phenotypic traits was seen in field trials in nine diverse field trial environments (Chapter 1, Section 4.3) and the introduced genes are not expected to alter traits related to weediness (apart from decreased pollen production and tolerance to glufosinate ammonium). Thus, it is highly unlikely that the introduced genes would confer increased weediness potential. The risk of increased weediness as a result of the introduced gene for tolerance to glufosinate ammonium is discussed extensively in four risk scenarios (Chapter 2, Sections 2.4.2–2.4.5). The effect of male sterility on pollen flow and outcrossing is discussed in Chapter 2, Sections 2.4.2 and 2.4.4.
	The RARMP should discuss the likelihood of seed dispersal and weediness of canola in RS3. Canola seed dispersal is unlikely to have a direct adverse environmental impact, as it does not persist beyond a few years in natural environments. The trait (HT) is unlikely to increase the weediness potential of GM canola or weedy relatives if gene flow occurs.	Noted.
	Risk scenarios in previous RARMPs of GM canola included discussion of potential causal pathways (e.g. seed dispersal) to harm, followed by assessment of that harm. In past Australian field trials, controls were put in place to minimise seed dispersal by wind, water, animals and birds. Recommends that RS3 include information on the causal pathway of seed dispersal that seed pod shattering, large seed numbers and the very small size of seeds mean that wind and water dispersal may be a problem and that there is no data on the relative importance of wind or water in seed dispersal. RS3 discusses seed spillage and dispersal by animals. RS3 could also note that, while seed spillage is the prime seed dispersal	Seed dispersal pathways, including spillage, small seed size and pod shattering are discussed throughout the RARMP (Chapter 1, Section 3; Chapter 2, Sections 2.4.2–2.4.5) and the associated biology document. As discussed in risk scenario 3 (Chapter 2, Section 2.4.3), there is no evidence that the introduced genes caused changes to seed dispersal characteristics of the GM canola, compared with non-GM canola

Submission	Summary of issues raised	Comment
	route, small seed size and shattering were also identified as a spontaneous dispersal route of canola seed in Canada.	
	While agrees canola is not a significant weed of natural environments in Australia (Par 191), it is a significant weed of agricultural areas. GM HT canola has emerged as a significant weed (of agricultural areas) in Argentina and Canada. Also crop transgenes have moved into truly wild populations for only three GM crops, one of which is HT canola. Given the recent evidence of dispersal and weediness, it is recommended that RS3 include discussion of weedy relatives in Australia and weediness of GM canola to support the conclusions that GM canola has a lower ability to spread and persist than non-GM canola.	Weedy relatives of canola are discussed in Chapter 1, Section 5.3.1. The risk of the GM canola outcrossing and hybridising with these species is considered in risk scenario 5 (Chapter 2, Sections 2.4.5). Risk scenario 2 (Chapter 2, Section 2.4.2) discusses how the male sterility trait reduces the ability for this GM canola to spread and persist. Discussion in this risk scenario has been updated to clarify that gene flow via pollen dispersal is highly unlikely in the GM canola.
6	Agrees that, overall, BASF Australia Ltd's application has negligible risks to the health and safety of people and the environment. Specifically, satisfied that the measures outlined in the Risk Assessment and Risk Management Plan (RARMP) for DIR 175 are adequate for managing the short and long-term risks from the proposal.	Noted.
7	Supported the Regulator's decision that DIR 175 poses negligible risk of harm to human and the environment.	Noted.
	Additional data should be provided on the impact of seed dispersal (e.g., via water flow) and abiotic stress (e.g., drought) tolerance of MS11 on the environment. This would enhance the RARMP's scientific rigour and build confidence in the community regarding the commercial release of MS11.	Submission 5 raised similar issues around seed dispersal and abiotic stress. See response to that submission (above) for further details. In summary, seed dispersal pathways are discussed throughout the RARMP and the associated biology document and there is no evidence that the introduced genes cause changes to seed dispersal characteristics of the GM canola, compared with non-GM canola. Similarly, the response of MS11 to abiotic stresses (including but not limited to drought stress) is similar to that of the non-GM counterpart.
	Agreed with the draft licence conditions but noted that the applicant has not proposed any measures to manage the seed dispersal of MS11, and further recommended the applicant to inform the Regulator should new information arise that may change this assessment.	For a commercial release, it is expected that the GM canola will be cultivated in the same manner as non-GM canola, with similar potential for seed dispersal. There is no evidence that the introduced genes cause changes to seed dispersal characteristics of the GM canola compared with non-GM canola, and the RARMP concluded that

Submission	Summary of issues raised	Comment
		there is negligible risk associated with the GM canola seed dispersal *per se*. Therefore, no specific measures to manage seed dispersal were included in the draft licence. The licence requires reporting of any unintended effects or additional information related to risks as a result of dealing with the GM canola.

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

The Regulator received two 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	That's just plain greedy and stupid!	Noted.
2	I oppose all suggestions to release genetically modified canola as a food for general consumption in Australia. Such a move would be highly undesirable and irreversible.	Food Standards Australia New Zealand (FSANZ) has regulatory responsibility for food safety assessments in Australia. FSANZ first approved food produced from a GM canola in 2000, and approved food derived from MS11 canola in 2017. More information about their assessments is available from the FSANZ website.