Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 139

Commercial release of canola genetically modified for herbicide tolerance

Applicant: Pioneer Hi-Bred Australia Pty Ltd

March 2016

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application DIR 139

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application for the intentional, commercial scale release of herbicide tolerant 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 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 proposed. However, general licence conditions have been imposed to ensure that there is ongoing oversight of the release.

The application

Application number	DIR 139
Applicant	Pioneer Hi-Bred Australia Pty Ltd (Pioneer)
Project title	Commercial release of canola genetically modified for herbicide tolerance[1]
Parent organism	*Brassica napus* L. (canola)
Introduced gene and modified trait	Glyphosate acetyltransferase (*gat4621*) gene derived from the bacterium *Bacillus licheniformis* for tolerance to the herbicide glyphosate
Proposed locations	Australia-wide
Primary purpose	Commercial release of the GM canola

Risk assessment

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

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 were 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 impact were considered.

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

[1] The title of the licence application submitted by Pioneer is “General release of canola genetically modified for optimal herbicide tolerance”.

The principal reasons for the conclusion of negligible risks are: the introduced protein is not considered toxic or allergenic to people or toxic to other desirable organisms; proteins similar to the introduced protein are widespread in the environment; the GM canola has been grown in field trials in Australia since 2012 without adverse or unexpected effects; the GM canola and its progeny can be controlled using integrated weed management; and the GM canola has limited capacity to establish in undisturbed 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 describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions.

As the level of risk is assessed as negligible, specific risk treatment is not required. However, the Regulator has imposed licence conditions 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.

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Abbreviations

Act	*Gene Technology Act 2000*
AMPA	Aminomethylphosphonic acid
APVMA	Australian Pesticides and Veterinary Medicines Authority
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
FDA	United States Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
g	Gram
gat4621	Glyphosate acetyltransferase gene
GAT4621	Glyphosate acetyltransferase enzyme
GM	Genetically modified
GMO	Genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
µmole	Micromole
m	Metre
mg	Milligram
mm	Millimetre
NAAsp	N-acetylaspartate
NAGlu	N-acetylglutamate
NAGly	N-acetylglycine
NASer	N-acetylserine
NAThr	N-acetylthreonine
ng	Nanogram
NSW	New South Wales
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
PCR	Polymerase chain reaction
pinII	Proteinase inhibitor II gene
Pioneer	Pioneer Hi-Bred Australia Pty Ltd
PRR	Post release review
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
RNA	Ribonucleic acid
TT	Triazine tolerant
UBQ10	Ubiquitin 10 gene
USDA	United States Department of Agriculture

Chapter 1 Risk assessment context

Section 1 Background

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

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

3. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

RISK ASSESSMENT CONTEXT

LEGISLATIVE REQUIREMENTS
(including Gene Technology Act and Regulations)

RISK ANALYSIS FRAMEWORK

OGTR OPERATIONAL POLICIES AND GUIDELINES

PROPOSED DEALINGS
Proposed activities involving the GMO
Proposed limits of the release
Proposed control measures

GMO
Introduced genes (genotype)
Novel traits (phenotype)

PREVIOUS RELEASES

PARENT ORGANISM
Origin and taxonomy
Cultivation and use
Biological characterisation
Ecology

RECEIVING ENVIRONMENT
Environmental conditions
Agronomic practices
Presence of related species
Presence of similar genes

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

Section 2 Regulatory framework

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and who must be consulted, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that inform the decisions on licence applications. In addition, the Regulations outline further matters the Regulator must consider when preparing a RARMP.

5. 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 given in Appendix A.

6. 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. One public submission was received and its consideration is summarised in Appendix C.

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

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

Section 3 The proposed release

9. Pioneer Hi-Bred Australia Pty Ltd (Pioneer) proposes commercial cultivation of GM canola that has been genetically modified for herbicide tolerance. The GM canola variety proposed for release is Optimum™ GLY Canola, which is also referred to as GM canola line DP73496 or by the OECD unique identifier DP-073496-4.

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

11. The dealings involved in the proposed intentional release are all dealings, *i.e.*:

(a) conducting experiments with the GMO

(b) making, developing, producing or manufacturing the GMO

(c) breeding the GMO with other canola cultivars

(d) propagating the GMO

(e) using the GMO in the course of manufacture of a thing that is not the GMO

(f) growing, raising or culturing the GMO

(g) importing the GMO

(h) transporting the GMO

[2] Pioneer is seeking approval for unrestricted commercial release of Optimum™ GLY Canola in all canola growing areas of Australia. Canola may be grown over a significant proportion of Australian agricultural land, including areas in all States. 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.

(i) disposing 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 4 The parent organism

12. The parent organism is *Brassica napus* L., which is commonly known as canola, rapeseed or oilseed rape. Canola is exotic to Australia and is grown as an agricultural crop mainly in Western Australia, New South Wales, Victoria and South Australia. It is Australia's third largest broad acre crop (ABARES 2015). Canola is primarily grown for its seed oil, which is used as a cooking oil and for other food and industrial applications. The seed meal which remains after oil extraction is used as animal feed (OECD 2011). Information on the weediness of the parent organism is summarised below and information on the use of the parent organism in agriculture is summarised in Section 6 (the receiving environment). More detailed information can be found in *The Biology of* Brassica napus *L. (canola)* (OGTR 2011), which was produced to inform the risk assessment process for licence applications involving GM canola plants and is available from the OGTR Risk Assessment References page.

13. The Standards Australia *National Post-Border Weed Risk Management Protocol* rates the weed risk potential of plants according to properties that strongly correlate with weediness for each relevant land use (Standards Australia Ltd 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). 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 2011). Please note that, because canola has been grown in Australia over several decades, its actual rather than potential distribution is addressed.

4.1 Potential to cause harm

14. In summary, 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
- limited potential to act as a reservoir for plant pests, pathogens or diseases.

15. *B. napus* seeds contain two natural toxicants: erucic acid and glucosinolates. 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 exclusively as livestock feed. The products of glucosinolate hydrolysis have negative effects on animal production (OECD 2011).

16. The term canola refers to varieties of *B. napus* that contain less than 2% erucic acid in the oil and less than 30 μmoles/g of glucosinolates in the seed meal, so are considered suitable for human and animal consumption (OECD 2011). The Australian canola crop grown in 2014 contained on average less than 0.1% erucic acid in the oil and approximately 12 μmoles/g of glucosinolates in the meal (Seberry et al. 2015).

4.2 Invasiveness

17. With regard to invasiveness, non-GM canola volunteers have:

- the ability to reproduce by seed, but not by vegetative means

- short time to seeding
- high annual seed production
- 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 from cropping areas and low potential for long distance spread by people from intensive land uses such as roadsides.

4.3 Actual distribution

18. In Australian agricultural settings, volunteer canola is considered to be a major problem warranting control (Groves et al. 2003). Canola volunteers requiring weed management are likely to be found in fields for up to three years after growing a canola crop (Australian Oilseeds Federation 2014; Salisbury 2002). Canola volunteers produce allelopathic compounds that reduce germination of other crops, in addition to directly competing with crop plants (Asaduzzaman et al. 2014; Gulden et al. 2008).

19. Feral canola plants are often observed growing on roadsides or railway easements in Australia; in the case of roadside canola 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 (Baker & Preston 2004; Crawley & Brown 2004; Gulden et al. 2008). 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 (OECD 2012; Salisbury 2002).

20. Canola is not considered a significant weed in natural undisturbed habitats in Australia (Dignam 2001; Groves et al. 2003).

Section 5 The GM canola

5.1 Introduction to the GMO

21. Optimum™ GLY Canola contains an introduced *gat4621*gene. The gene encodes a glyphosate acetyltransferase enzyme which confers tolerance to the herbicide glyphosate. The *gat4621* gene was derived from three *gat* genes isolated from *Bacillus licheniformis* and optimised to create an enzyme with higher efficiency and increased specificity for glyphosate.

22. Short regulatory sequences that control expression of the introduced gene are also present in the GM canola line. These sequences are derived from *Arabidopsis thaliana* (thale cress) and *Solanum tuberosum* (potato).

5.2 The introduced gene, its encoded protein and associated effects

5.2.1 The gat4621 gene, its protein and end products

23. The *gat4621* gene is derived from *B. licheniformis*, a common gram positive soil bacterium. The gene encodes a glyphosate *N*-acetyltransferase enzyme (GAT), which belongs to a family of *N*-acetyl transferases known as the GNAT superfamily (Castle et al. 2004). Enzymes in this superfamily occur widely in plants, animals and microbes and have low sequence homology but a highly conserved tertiary structure. GNAT enzymes use acetyl-CoA as an acetyl donor to acetylate a variety of substrates (Vetting et al. 2005).

24. The *gat4621* gene in Optimum™ GLY Canola is expected to confer tolerance to herbicides containing glyphosate (*N*-phosphonomethyl glycine). Glyphosate is the active ingredient in a number of broad-spectrum systemic herbicides that have been approved for use in Australia (APVMA website). The herbicidal activity of glyphosate is derived from its ability to bind to and inhibit the function of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme involved in the shikimate biosynthetic pathway in plants. Glyphosate binding effectively terminates the shikimate pathway prematurely, preventing biosynthesis of essential aromatic amino acids, and eventually leading to cell death (Dill 2005).

25. The most widely used approach to conferring glyphosate tolerance in GM plants has been the introduction of EPSPS variants that are insensitive to glyphosate inhibition. GAT proteins provide an alternative mechanism of glyphosate tolerance by acetylating the secondary amine of glyphosate to produce *N*-acetyl glyphosate. This is a larger molecule than glyphosate and is unable to bind effectively to the active site of EPSPS. The normal activity of EPSPS is not inhibited and consequently the herbicide is no longer phytotoxic (Castle et al. 2004; Siehl et al. 2007).

26. Residue analysis of Optimum™ GLY Canola seed from Canadian field trials showed that the main herbicide residues present were *N*-acetyl glyphosate and unaltered glyphosate. The metabolites aminomethylphosphonic acid (AMPA) and *N*-acetyl aminomethylphosphonic acid (*N*-acetyl AMPA), shown in Figure 2, were also detected. AMPA was less than 1% and *N*-acetyl AMPA was less than 2% of total residues. Herbicide residues were not detectable in refined canola oil (FSANZ 2014).

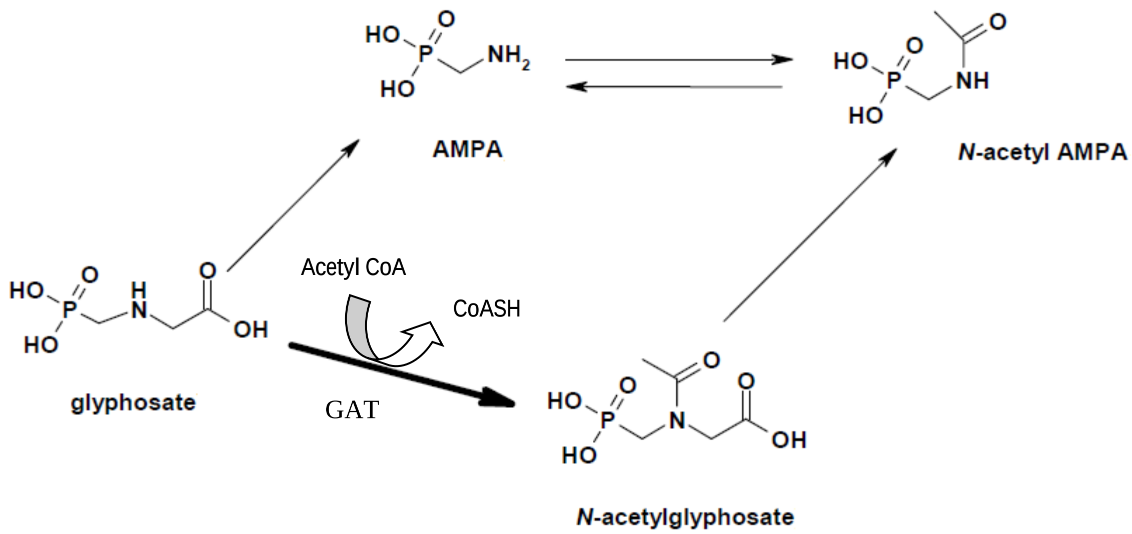

Figure 2. Enzymatic activity of GAT4621 in GM plants

27. The *gat4621* gene is a synthetic gene obtained by a directed evolution process. The starting point was three *gat* genes with low activity towards glyphosate derived from three different strains of *B. licheniformis* (B6, 401 and DS3). The native *gat* genes were subjected to a gene shuffling process, using fragmentation and recombination to generate libraries of gene variants, which were screened for glyphosate acetylation. Progeny with improved properties were used as parents for the next round of shuffling. The *gat4621* gene was obtained after eleven rounds of gene shuffling including two rounds where genetic diversity was introduced via the polymerase chain reaction (PCR) (Castle et al. 2004). The GAT4621 enzyme showed a 7000-fold increase in catalytic efficiency towards glyphosate compared to

the native GATs (Siehl et al. 2005). The activity of GAT4621 with glyphosate as a substrate is comparable to the activity of other *N*-acetyltransferases with their natural substrates (Siehl et al 2007).

28. The *gat4621* gene was optimised for expression in plants by codon substitutions and one codon addition. The GAT4621 protein sequence is 75-78% identical and 90-91% similar to the sequences of each of the three native *B. licheniformis* GAT proteins.

29. A substrate specificity study was conducted by the applicant to determine whether the GAT4621 enzyme acetylated substrates other than glyphosate. Twenty agrochemicals, 21 amino acids and 11 antibiotics with structural similarities to glyphosate were tested as potential substrates. Measurable enzyme activity was detected only for five amino acid substrates: L-aspartate, L-glutamate, L-serine, L-threonine and glycine. The catalytic efficiency of GAT4621 towards L-aspartate, L-glutamate, L-serine, and L-threonine was about 1%, 0.8%, 0.05% and 0.06%, respectively, of the efficiency towards glyphosate. The catalytic efficiency towards glycine was too low to be determined (Larson et al. 2011).

30. The levels of the acetylated forms of the five amino acid substrates were measured in Optimum™ GLY Canola seed and control non-GM canola seed in Canadian and US field trials (Table 1). All acetylated amino acids were present in non-GM canola seed at low levels. The levels of N-acetylglutamate (NAGlu) and N-acetylaspartate (NAAsp) were much higher in the GM seed than in the control seed, and above the range found in commercial varieties of non-GM canola. The levels of N-acetylthreonine (NAThr) and N-acetylserine (NASer) were higher in the GM seed than in the control seed but within the range found in commercial canola varieties. The levels of N-acetylglycine (NAGly) in GM seed and control seed were not statistically different. No acetylated amino acids were detected in refined canola oil derived from Optimum™ GLY Canola (FSANZ 2014).

Table 1. Concentrations of acetylated amino acids in canola seed

Analyte	Mean concentration in control canola (µg/g dry weight)	Mean concentration in GM canola (µg/g dry weight)	P-value	Tolerance interval containing 99% of commercial canola (µg/g dry weight)
NAAsp	1.24	1480	<0.0001	0.00861 – 4.43
NAGlu	0.628	32.8	<0.0001	0.0968 – 5.37
NAGly	0.0751	0.0825	0.454	0.0240 – 0.338
NASer	0.843	1.04	0.0035	0.0524 – 27.2
NAThr	0.110	0.546	<0.0001	0.0140 – 1.74

5.2.2 Toxicity and allergenicity of the GAT4621 protein

31. The three genes used to construct the synthetic GAT4621 gene were derived from *B. licheniformis*, which is a widely distributed soil bacterium (Rey et al. 2004). The American Type Culture Collection classifies this bacterium as Biosafety Level 1, for organisms that are not known to cause disease in healthy adults (American Type Culture Collection website; accessed November 2015). *B. lichenformis* also has a history of safe use in the food industry, as the species is used to produce enzymes as food processing aids (Rey et al. 2004).

32. An acute oral toxicity study where a single dose of 1.6 g/kg of purified GAT4621 was fed to mice produced no signs of acute toxicity (Rood 2007). A sequence similarity search recently performed by the applicant found that the GAT4621 protein had no sequence matches to proteins that are known toxins. A thirteen week subchronic feeding study where

meal and oil derived from Optimum™ GLY Canola were fed to rats as approximately 22% of their diet found no toxicologically significant differences between the groups fed GM canola, either glyphosate-treated or untreated, and the control groups fed non-GM canola (Delaney et al. 2014). A six week nutritional equivalency study where broiler chickens were fed canola meal derived from Optimum™ GLY Canola as 10-20% of their diet found no differences in growth performance between groups whose diet contained GM canola, either glyphosate-treated or untreated, and control groups whose diet contained non-GM canola (McNaughton et al. 2014).

33. The protein sequence of GAT4621 was compared to a database of allergens from the Food Allergy Research and Resource Program (updated 2014), and no similarity was found to any known protein allergen. Typically, food proteins that are allergenic tend to be stable to digestive enzymes. *In vitro* digestibility of GAT4621 was assessed, and it was found to be completely degraded within 30 seconds when incubated with pepsin and within five minutes when incubated with pancreatin (Rood 2007).

34. FSANZ has assessed and approved the safety of food derived from both canola and maize expressing the GAT4621 protein. FSANZ concluded that there is strong evidence that GAT4621 is unlikely to be toxic or allergenic in humans (FSANZ 2010; FSANZ 2014).

5.2.3 Toxicity of metabolites

35. The potential toxicity of herbicide metabolites will be considered by the APVMA in its assessment of Pioneer’s application regarding herbicide treatments for Optimum™ GLY Canola. FSANZ previously conducted a hazard assessment of glyphosate residues for a GM soybean variety expressing a GAT protein homologous to GAT4621. FSANZ concluded that N-acetyl glyphosate, N-acetyl AMPA and AMPA are all less toxic than glyphosate (FSANZ 2009).

36. A number of toxicology studies have been conducted on the five acetylated amino acids NAAsp, NAGlu, NAThr, NASer and NAGly. None of the studies showed evidence of adverse effects, except acute toxicity of N-acetylaspartate to rats at the highest dose of 5 g/kg body weight, where unmodified aspartate is also toxic (Larson et al. 2011). The acetylation of amino acids occurs naturally as a common post-translational modification of proteins and peptides in eukaryotes (Polevoda & Sherman 2002). Acetylation of amino acids is also used to improve the functional properties of proteins for food processing (El-Adawy 2000). Thus, acetylated amino acids are normal constituents of human and animal diets. Mammals have a variety of acylase enzymes that remove the acetyl group from acetylated amino acids to regain the free amino acid (Perrier et al. 2005).

37. It is possible the GAT4621 enzyme could acetylate non-standard amino acids or other substrates with structural similarities to glyphosate that were not tested by the applicant in its substrate specificity study (Larson et al. 2011). However, this is unlikely to lead to any toxicity, because as discussed in Section 5.2.2, a subchronic feeding study of Optimum™ GLY Canola meal and oil to rodents did not detect toxicity (Delaney et al. 2014) , and a poultry feeding study found that Optimum™ GLY Canola meal was nutritionally equivalent to non-GM canola meal (McNaughton et al. 2014).

38. As described in Section 4.1.1, canola seeds naturally contain glucosinolates, which are toxicants. GAT4621, which is an amino acid acetyltransferase, is not expected to alter the metabolic pathways for synthesis of glucosinolates. The total glucosinolate concentration for Optimum™ GLY Canola seeds was 5.7 µmoles/g dry weight, compared to an average 6.4 µmoles/g dry weight in the Australian canola crop (Seberry et al. 2015). Compositional

analysis demonstrated that the concentrations of various glucosinolates in the GM canola were comparable to concentrations in non-GM canola.

39. The FSANZ assessment of Optimum™ GLY Canola concluded that food derived from the GM canola is as safe for human consumption as food derived from non-GM canola (FSANZ 2014).

5.3 The regulatory sequences

40. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct gene transcription. Expression of the *gat4621* gene in the GM canola is controlled by the promoter region of the ubiquitin 10 (*UBQ10*) gene from *Arabidopsis thaliana* (thale cress). The promoter region includes the promoter, the 5' untranslated region, and the 5' intron (Norris et al. 1993). The *UBQ10* promoter leads to constitutive expression in all plant parts.

41. Also required for gene expression in plants is a messenger RNA termination region, including a polyadenylation signal. The terminator used in the GM canola line is the 3' terminator sequence from the proteinase inhibitor II (*pinII*) gene of *Solanum tuberosum* (potato) (An et al. 1989; Keil et al. 1986).

42. The regulatory sequences are derived from plants that are widespread in the environment. Humans, animals and other organisms are commonly exposed to these plants.

5.4 Method of genetic modification

43. The GM canola was generated using biolistic transformation of canola microspores with the gel-purified DNA fragment PHP28181A (Table 2). Information about biolistic transformation can be found in the document *Methods of plant genetic modification* available from the Risk Assessment References page on the OGTR website.

Table 2. Description of the genetic elements contained in DNA fragment PHP28181A

Genetic element	Location on transforming DNA fragment (size, bp)
Polylinker sequence	1 – 7 (7)
UBQ10 promoter	8 – 1312 (1305)
Intervening sequence	1313 – 1335 (23)
gat4621	1336 – 1779 (444)
Intervening sequence	1780 – 1796 (17)
pinII terminator	1797 – 2106 (310)
Polylinker sequence	2107 – 2112 (6)

44. After transformation, plant cells were cultured in the presence of glyphosate, and subsequently shoots were grown in growth medium supplemented with glyphosate, to select for glyphosate tolerance. Regenerated plants were evaluated and Optimum™ GLY Canola was identified as the line with the preferred molecular and phenotypic characteristics.

45. The parental canola used for genetic modification was 1822B, a commercial Canadian canola line. For Australian release, the GM canola event was introgressed into commercial Australian canola lines. The Australian hybrids for release also contain a non-GM trait for tolerance to imidazolinone herbicides.

5.5 Characterisation of the GMO

5.5.1 Molecular characterisation

46. Southern blot analysis provided by the applicant demonstrated that a single, intact copy of the PHP28181A DNA fragment was inserted into the GM canola genome. PCR analysis found that the insert sequence was identical to the sequence of DNA fragment PHP28181A except for a deletion of base pairs 1-3, which are part of a polylinker sequence that would not be expressed (see Table 2). Therefore, the deletion is not expected to affect the function of the insert.

47. DNA fragment PHP28181A was isolated from a parent plasmid. Southern blot analysis conducted by the applicant determined that no plasmid sequence except the intended DNA fragment was present in the GM canola. PCR analysis of the flanking genomic regions of the insert confirmed that these regions are of canola origin. The site of integration of the inserted DNA within the host genome is not known.

48. Southern blot analysis of five generations of the GM canola showed that the insert was stably inherited at a single genetic locus. Segregation analysis of four generations found segregation ratios consistent with the expected Mendelian inheritance.

5.5.2 Levels of GAT4621 protein expression

49. Expression levels of GAT4621 protein in Optimum™ GLY Canola were measured by the applicant using a quantitative enzyme-linked immunosorbent assay (ELISA). GAT4621 concentration in whole plants (above ground portion), over three different developmental stages and at six different field trial sites in North America, ranged between 3.1 – 10 ng/mg dry weight. GAT4621 concentration in GM canola roots averaged 6.6 ng/mg dry weight. GAT4621 concentration in GM canola seeds averaged 6.2 ng/mg dry weight. Compositional analysis of the GM canola seeds found that they averaged 25.9% protein on a dry weight basis, so GAT4621 comprises approximately 0.002% of total seed protein.

5.5.3 Phenotypic and agronomic characterisation

50. The agronomic performance of Optimum™ GLY Canola was assessed in field trials in Canada and the United States. The phenotypic characteristics measured were early population, seedling vigour, early growth, plant height, lodging, shattering, final population, days to flowering, flowering duration, days to maturity and yield. There were no statistically significant differences in these characteristics between Optimum™ GLY Canola and near-isoline non-GM canola, except that in one experiment the time to flowering of Optimum™ GLY Canola was one day longer than the control canola, but within the normal range of time to flowering for other canola varieties. The difference was not replicated in a repeat of the experiment (Larson et al. 2011).

51. The agronomic performance of Optimum™ GLY Canola was assessed in Australian field trials over two years at six sites in Victoria, NSW and Western Australia (see Figures 3, 4, 5 and 6). The disease incidence and insect damage in GM and non-GM control canola were also assessed in these field trials (see Figures 5 and 6). For most characteristics, Optimum™ GLY Canola treated with glyphosate appeared to have very similar performance to the control canola. For six out of ten experiments, the time to flowering of the GM canola was several days longer than the control canola, with a corresponding increase in time to maturity. It is not clear whether this difference is statistically significant, but in any case, the altered characteristic would not be expected to lead to increased weediness. In all ten experiments, the seedling vigour of the GM canola was lower than the control canola, by an average of approximately half a point on a scale of 1-9. Again, even if this difference is statistically significant, the altered characteristic would not be expected to lead to increased weediness.

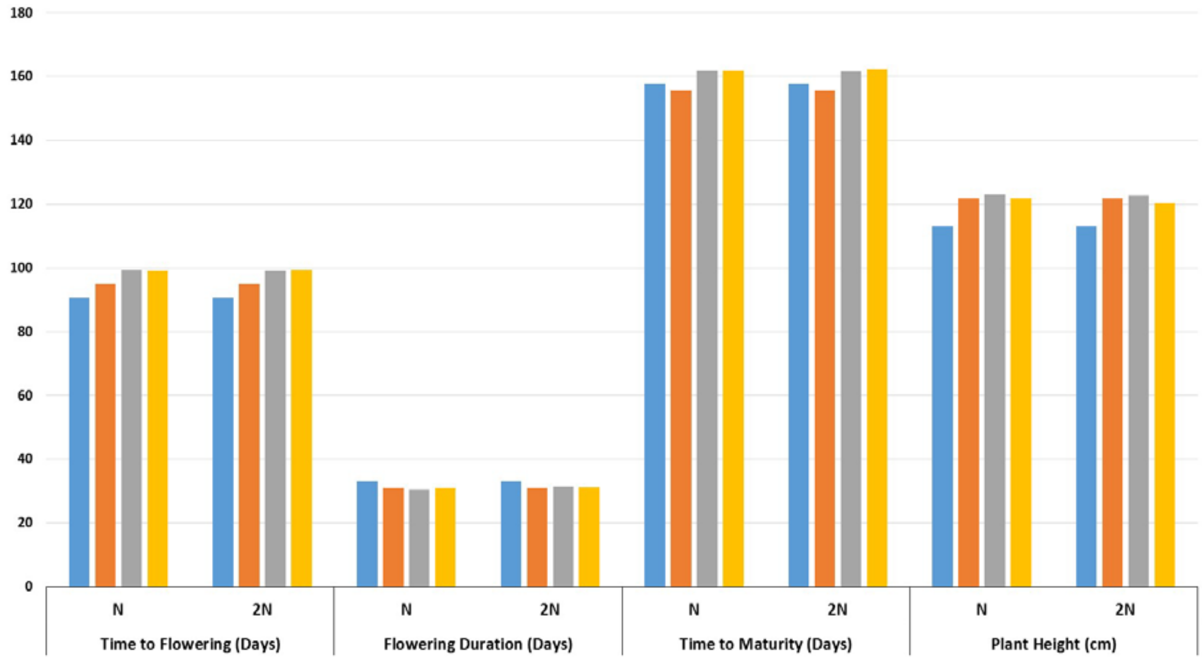

Figure 3. Agronomic characteristics measured in Australian field trials in 2013. Blue: Hyola 50 comparison canola; orange: isoline non-GM control canola; grey: Optimum™ GLY Canola treated once with glyphosate; yellow: Optimum™ GLY Canola treated twice with glyphosate. N and 2N indicate rates of herbicide application.

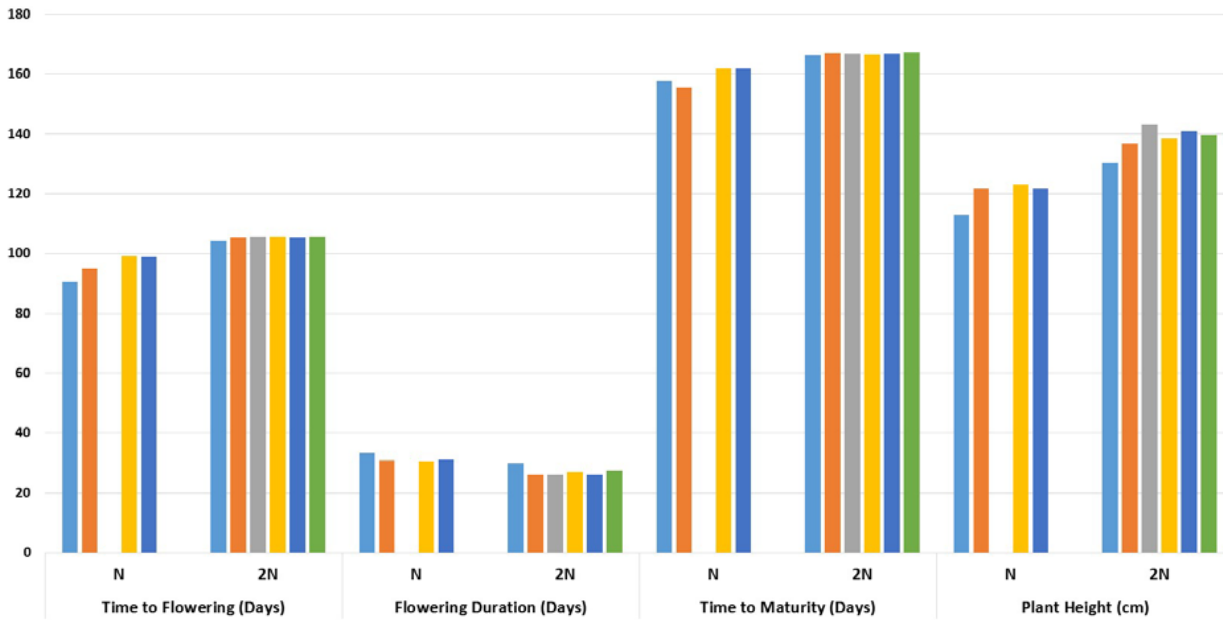

Figure 4. Agronomic characteristics measured in Australian field trials in 2014. Light blue: Hyola 50 comparison canola; orange: isoline non-GM control canola; grey and yellow: Optimum™ GLY Canola treated once with glyphosate; dark blue: Optimum™ GLY Canola treated twice with glyphosate; green: Optimum™ GLY Canola treated three times with glyphosate. N and 2N indicate rates of herbicide application.

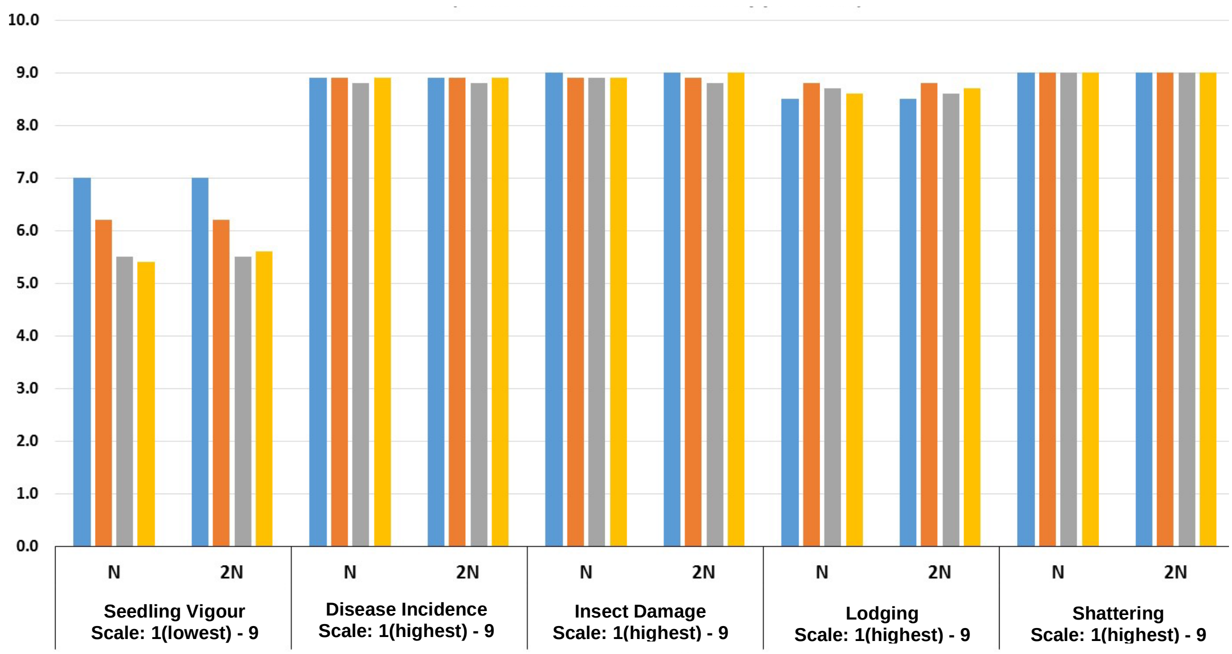

Figure 5. Agronomic and environmental interaction characteristics measured in Australian field trials in 2013. Blue: Hyola 50 comparison canola; orange: isoline non-GM control canola; grey: Optimum™ GLY Canola treated once with glyphosate; yellow: Optimum™ GLY Canola treated twice with glyphosate. N and 2N indicate rates of herbicide application.

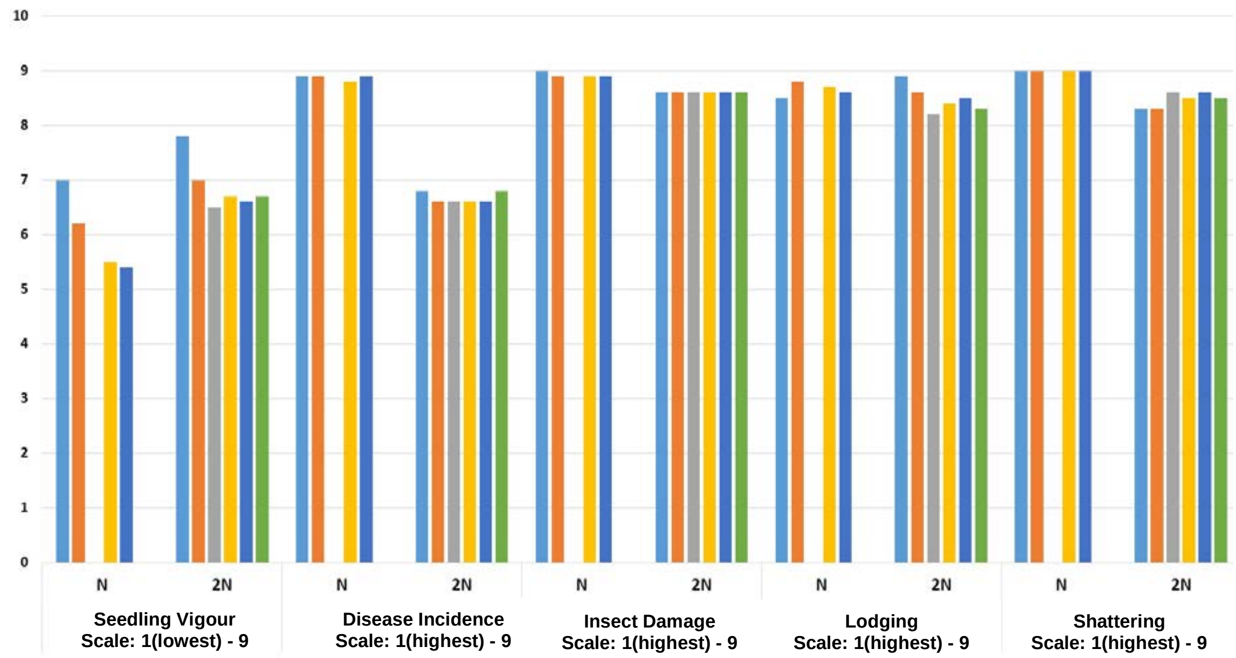

Figure 6. Agronomic and environmental interaction characteristics measured in Australian field trials in 2014. Light blue: Hyola 50 comparison canola; orange: isoline non-GM control canola; grey and yellow: Optimum™ GLY Canola treated once with glyphosate; dark blue: Optimum™ GLY Canola treated twice with glyphosate; green: Optimum™ GLY Canola treated three times with glyphosate. N and 2N indicate rates of herbicide application.

52. Seed germination data for Optimum™ GLY Canola were collected in laboratory experiments. There was no statistically significant difference ($P < 0.05$) in germination rates between GM canola and non-GM control canola under cold conditions or diurnal conditions with cycling temperatures. The GM canola had a slightly lower mean germination rate than the control canola under warm conditions, but this germination rate was within the range found in reference commercial canola varieties (Larson et al. 2011).

53. The applicant carried out compositional analysis of Optimum™ GLY Canola seed grown in five North American field trial sites, in comparison to a near-isoline non-GM control and reference non-GM commercial canola varieties. Concentrations were measured for 85 analytes, which were selected according to an international consensus document on compositional considerations for canola (OECD 2001). For most analytes, there was no statistically significant difference between concentrations in GM canola seed and in near-isoline control canola seed. Statistically significant differences ($P < 0.05$) were found for a few analytes, with GM canola having reduced concentrations of oleic acid, magnesium and the glucosinolate progoitrin and increased concentrations of linoleic acid, delta- and total tocopherols, and cholesterol compared to control non-GM canola. However, the concentrations for each of these analytes were within the tolerance intervals calculated for commercial non-GM canola varieties. FSANZ assessed this compositional analysis and found that the data support the conclusion that there are no biologically significant differences in the levels of key constituents in seed from Optimum™ GLY Canola compared with non-GM canola (FSANZ 2014).

Section 6 The receiving environment

54. 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).

55. The applicant has proposed to release Optimum™ GLY 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. Canola growing areas are mainly in the Australian winter cereal belt of NSW, Victoria, South Australia, and Western Australia. Small quantities of canola are grown in Southern Queensland and Tasmania (OGTR 2011). The actual locations, number of sites and area of land used in the proposed release would depend on factors such as field conditions, grower demand and seed availability.

6.1 Relevant agronomic practices

56. In Australia, canola is commonly grown in rotation with wheat as the following crop. 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, phosphorus and sulphur than most other crops so fertiliser application is important. Canola is harvested either by windrowing (swathing) or less commonly by direct harvesting. Windrowing involves cutting the crop and placing it in rows to dry. After 1-2 weeks, when most of the seed has matured and the moisture content is under 9%, the windrow is picked up by the harvester. Standard cultivation practices for canola are

discussed in more detail in *The Biology of* Brassica napus *L. (canola)* (OGTR 2011) and *Canola best practice management guide for south-eastern Australia* (GRDC 2009).

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

58. The applicant is developing a Crop Management Plan that farmers growing Optimum™ GLY Canola will be required to follow.

6.2 Relevant abiotic factors

59. 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 dominant winter rainfall environments that receive more than 400 mm rainfall per year. 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, which restricts root development (GRDC 2009; Walton et al. 1999).

60. Other abiotic stresses that can reduce canola yields include frost, particularly during early pod development, and heat stress (GRDC 2009). Additional information regarding 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)* (OGTR 2011).

6.3 Relevant biotic factors

61. 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. Blackleg is managed by choosing cultivars 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; Howlett et al. 1999).

62. Canola is most susceptible to insect pests during establishment of the crop, at which time earth mites, lucerne flea and false wireworms cause the greatest damage. Damage can also be caused by aphids, native budworm and Rutherglen bug from flowering to podding (GRDC 2009; Miles & McDonald 1999).

63. Canola is highly susceptible to weed competition during the early stages of growth. The most problematic weeds include grassy weeds, such as annual ryegrass, vulpia and wild oat, volunteer cereals, and weeds from the *Brassicaceae* family, which can also reduce product quality through seed contamination. The most detrimental *Brassicaceae* weeds are wild radish (*Raphanus raphinastrum*), Indian hedgemustard (*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 (*Hirschfeldia incana*) (GRDC 2009; Sutherland 1999).

6.4 Presence of related plants in the receiving environment

64. Canola is predominantly self-pollinating, with an average of around 70% of canola seeds resulting from self-fertilisation. However, outcrossing between canola plants can be mediated by insects, wind or physical contact. Outcrossing frequencies between adjacent fields are highest in the first 10 m of the recipient fields, with rates averaging about 1.8% over this area, and rates decline with distance (Husken & Dietz-Pfeilstetter 2007). Under Australian conditions, a large study found that outcrossing rates between neighbouring commercial canola fields were less than 0.1% averaged over whole fields (Rieger et al. 2002).

65. 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 Australia canola crop comprised approximately 60% triazine tolerant (TT), 15% imidazolinone tolerant (Clearfield®), 20% Roundup Ready® and 5% non-herbicide tolerant canola varieties (Nick Goddard, Australian Oilseeds Federation, personal communication, 2015).

66. TT canola varieties were obtained by conventional breeding and have tolerance to Group C triazine herbicides. TT canola was the first type of herbicide tolerant canola introduced to Australia, and became very popular despite a significant yield penalty associated with the trait (Pritchard 2014).

67. Clearfield® canola varieties are conventionally bred and have tolerance to Group B imidazolinone herbicides. The Clearfield® trait is also available in Juncea canola (*Brassica juncea* or Indian mustard, discussed below) (DPI NSW 2013).

68. Roundup Ready® canola varieties are genetically modified and were approved for commercial release by the Regulator (DIR 020/2002). They have tolerance to glyphosate herbicide (Group M) but by a different mechanism to Optimum™ GLY Canola. Dual herbicide tolerant RT® canola, which is a cross between Roundup Ready® and TT canola, was released in 2015 (Pacific Seeds website). TruFlex™ Roundup Ready® canola, a newer variant of Roundup Ready® canola, has been approved for commercial release by the Regulator (DIR 127), but has not yet entered commercial production in Australia.

69. GM InVigor® canola, which has tolerance to Group N glufosinate herbicide, was approved for commercial release by the Regulator either alone (DIR 021/2003) or combined with Roundup Ready® canola (DIR 108). However, these canola varieties have only been grown on a limited scale for breeding work and not yet entered commercial production in Australia.

70. Canola can cross with other *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 oilseed canola (OGTR 2011).

71. Canola can spontaneously cross with the related crop species *B. juncea* (Indian mustard or Juncea canola) and *B. rapa* (including turnips) (Liu et al. 2010; Warwick et al. 2003), and there is one report of field crosses with the crop species *B. oleracea* (including cabbage, cauliflower and broccoli) (Ford et al. 2006). Juncea canola (*B. 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 2009). Horticultural crops that are variants or subspecies of *B. napus*, *B. juncea*, *B. rapa* or *B. oleracea* are also commercially grown in Australia.

72. Under open pollination conditions, naturally occurring hybrids between *B. napus* and the related weedy species *Raphanus raphanistrum* and *Hirschfeldia incana* have been reported at very low frequencies (Darmency & Fleury 2000; Darmency et al. 1998). According to the Australian Department of the Environment, *R. raphanistrum* (wild radish) is a serious agricultural weed widespread in all states and territories except the Northern Territory. *H. incana* (Buchan weed) is a common roadside weed found in Queensland, NSW, Victoria, Tasmania and South Australia (National weeds lists; accessed November 2015).

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

73. The introduced *gat4621* gene is a composite of three *gat* genes derived from three different strains of *B. licheniformis* (see Section 5.2). The *gat4621* gene is synthetic as its sequence has been significantly modified to optimise its function. The synthetic gene and its encoded protein are not present in the Australian environment. However, similar *gat* genes are present in the Australian environment as *B. licheniformis* is a common soil bacterium, and thus humans and other organisms would commonly encounter its genes and their encoded proteins.

Section 7 Previous releases

7.1 Australian approvals of the GM canola line

74. Optimum™ GLY Canola was approved by the Regulator for limited and controlled release under licence DIR 114 and has been grown in field trials in NSW, Victoria and Western Australia since 2012. The Regulator has not received any report of adverse effects as a result of this release.

7.2 Approvals by other Australian agencies

75. 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.

76. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has approved the use of food derived from Optimum™ GLY Canola (herbicide-tolerant canola line DP-073496-4). This approval is listed in the Schedule to Standard 1.5.2 of the Australia New Zealand Food Standards Code. FSANZ has determined that food derived from the GM canola is as safe for human consumption as food derived from conventional (non-GM) canola varieties (FSANZ 2014).

77. The APVMA has regulatory responsibility for agricultural chemicals, including herbicides and insecticidal products, in Australia. Pioneer has submitted an application to the APVMA regarding herbicide treatments for Optimum™ GLY Canola.

7.3 International approvals

78. In Canada and the United States Optimum™ GLY Canola has been approved for commercial cultivation and in a number of countries products derived from Optimum™ GLY Canola have been approved for human food and animal feed use (Table 3). Optimum™ GLY Canola is not yet cultivated as a commercial crop in any country.

Table 3. International approvals of Optimum™ GLY Canola

Country	Authority	Type of Approval	Date
Canada	CFIA	Cultivation and feed	2012
Canada	Health Canada	Food	2012
Japan	MHLW	Food	2014
Japan	MAFF	Feed	2015
Mexico	COFEPRIS	Food and feed	2012
Republic of Korea	MOTIE	Food and feed	2015
United States	FDA	Food and feed	2012
United States	USDA	Cultivation	2013

Chapter 2 Risk assessment

Section 1 Introduction

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

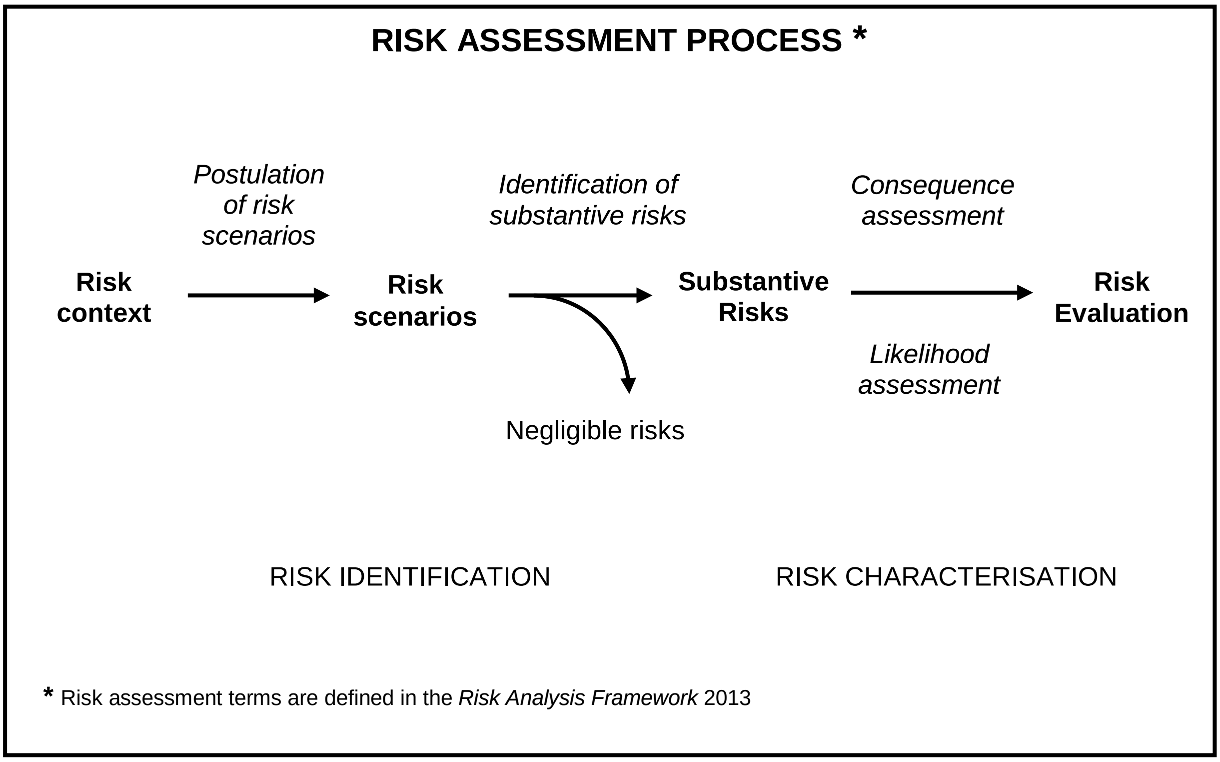

Figure 7 The risk assessment process

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

81. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, reported international experience and consultation (OGTR 2013). 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.

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

83. Substantive risks (*i.e.* those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). Risk evaluation then combines the Consequence and Likelihood assessments to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 Risk identification

84. Postulated risk scenarios are comprised of three components:

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

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

iii. Potential harm to an object of value: people or the environment.

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

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

2.1 Risk source

86. 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.

87. As discussed in Chapter 1, the GM canola proposed for release has been modified by the introduction of one glyphosate herbicide tolerance gene. This introduced gene, its encoded protein and resultant metabolites are considered further as a potential source of risk.

88. As discussed in Chapter 1, Section 5.3, the introduced gene is controlled by introduced regulatory sequences. These regulatory sequences are derived from common plants. Regulatory sequences are naturally present in plants, and the introduced elements are expected to operate in similar ways to endogenous elements. The regulatory sequences are DNA that is not expressed as a protein, and dietary DNA has no toxicity (Society of Toxicology 2003). Hence, potential harms from the regulatory elements will not be considered further. However, the introduced regulatory sequences control gene expression and hence the distribution and concentration of the introduced protein in the GM plants. The effects of protein levels, especially in relation to toxicity and allergenicity, will be considered below.

89. 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 and in plants generated by conventional breeding (Bradford et al. 2005; Ladics et al. 2015; Schnell et al. 2015). 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 (Bradford et al. 2005; Steiner et al. 2013). Therefore, unintended effects resulting from the process of genetic modification will not be considered further.

2.2 Causal pathway

90. 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.

91. Although all of these factors are taken into account, some are not included in risk scenarios because they are regulated by other agencies or have been considered in previous RARMPs (see sections 2.2.1 to 2.2.3, below).

2.2.1 Agronomic management and development of herbicide resistance

92. 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[3], increases the likelihood of selecting weeds that have developed herbicide resistance through natural mechanisms (Gressel 2002). This is not a novel issue associated with this GMO, as most canola currently grown in Australia is herbicide tolerant, by either non-GM or GM mechanisms (see Chapter 1, Section 6.4).

93. The genetic modification to Optimum™ GLY Canola confers tolerance to glyphosate, which is a widely used herbicide in Australia. A number of glyphosate resistant weed populations have already been identified in Australia. The weed species reported include *Brachiaria eruciformis*, *Bromus diandrus*, *Bromus rubens*, *Chloris truncata*, *Conyza bonariensis*, *Echinochloa colona*, *Lolium rigidum*, *Raphanus raphanistrum*, *Sonchus oleraceus* and *Urochloa panicoides* (The international survey of herbicide resistant weeds; accessed November 2015).

94. 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

[3] 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 tolerance in weeds.

and sets their conditions of use. Pioneer has submitted an application to the APVMA regarding herbicide treatments for Optimum™ GLY Canola. The APVMA will consider the potential for development of herbicide resistance prior to changing the relevant herbicide product labels to include appropriate use of glyphosate on the GM canola. Therefore, the issue of development of herbicide resistant weeds through selective pressure will not be further considered in this risk assessment.

2.2.2 Gene transfer by horizontal gene transfer

95. The potential for horizontal gene transfer from GMOs to other species that are not sexually compatible, and any possible adverse outcomes, has been reviewed in the scientific literature (Keese 2008) as well as assessed in many previous RARMPs. Horizontal gene transfer was most recently considered in detail in the RARMP for DIR 108. This and other RARMPs are available from the GMO Record on the OGTR website or by contacting the OGTR. In previous assessments of horizontal gene transfer no substantive risk was identified, due to the rarity of these events and because similar gene sequences are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, horizontal gene transfer will not be assessed further.

2.2.3 Unauthorised activities

96. The potential for unauthorised activities to lead to harm has been considered in previous RARMPs. In previous assessments of unauthorised activities, no substantive risk was identified. The Act provides for substantial penalties for unauthorised dealings with GMOs or non-compliance with licence conditions, and also requires the Regulator to have regard to the suitability of an applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, unauthorised activities will not be considered further.

2.3 Potential harm

97. 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).

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

2.4 Postulated risk scenarios

99. Seven risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 4 and discussed individually below. 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 the commercial use or the spread and persistence of plant material.

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

Table 4 Summary of risk scenarios from the proposed dealings

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced gene for herbicide tolerance	Commercial cultivation of GM canola expressing the herbicide tolerance gene ⬇ Exposure of people to the introduced protein and resultant metabolites by consumption of oil from GM canola, inhalation of GM canola pollen, or occupational contact with GM canola plants or products	Increased toxicity or allergenicity for people	No	• The GAT4621 protein and resultant metabolites are not toxic or allergenic to people. • The GAT4621 protein and resultant metabolites are not present at detectable levels in oil from GM canola. • FSANZ has approved products derived from the GM canola for use in human food.
2	Introduced gene for herbicide tolerance	Commercial cultivation of GM canola expressing the herbicide tolerance gene ⬇ Exposure of desirable organisms to the introduced protein and resultant metabolites through consumption of GM canola plants or products or contact with GM canola plants	Increased toxicity for desirable organisms	No	• The GAT4621 protein and resultant metabolites are not toxic to mammals or birds. • The GM canola seed is compositionally equivalent to non-GM canola seed. • Proteins similar to GAT4621 are widespread in the environment.
3	Introduced gene for herbicide tolerance	Commercial cultivation of GM canola in agricultural areas ⬇ Establishment of volunteer GM canola plants expressing the herbicide tolerance gene in agricultural areas ⬇ Reduced effectiveness of weed management measures to control the volunteers	Reduced establishment or yield of desirable agricultural crops or Increased reservoir for pests and pathogens	No	• Integrated weed management practices would effectively control GM canola volunteers in agricultural areas.
4	Introduced gene for herbicide tolerance	Commercial cultivation of GM canola in agricultural areas ⬇ Dispersal of GM canola seed to intensive use areas ⬇ Establishment of feral GM canola plants expressing the herbicide tolerance gene in intensive use areas ⬇ Reduced effectiveness of weed management measures to control the feral plants	Reduced services from the land use or Reduced biodiversity	No	• Canola is not a persistent weed in intensive use areas. • Weed management strategies other than glyphosate use can control feral GM canola. • Most land managers of intensive use areas where feral canola is present do not consider that canola warrants management.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
5	Introduced gene for herbicide tolerance	Commercial cultivation of GM canola in agricultural areas ⬇ Dispersal of GM canola seed to nature reserves ⬇ Establishment of feral GM canola plants expressing the herbicide tolerance gene in nature reserves ⬇ Reduced effectiveness of weed management measures to control the feral plants	Reduced establishment of desirable native vegetation or Reduced services from the land use	No	• Canola is not a significant weed in nature reserves. • The introduced gene does not increase the potential weediness of the GM canola.
6	Introduced gene for herbicide tolerance	Commercial cultivation of GM canola in agricultural areas ⬇ Cross-pollination with other canola, including canola with other herbicide tolerance traits ⬇ Establishment of hybrid GM canola plants expressing the herbicide tolerance gene as volunteers ⬇ Reduced effectiveness of weed management measures to control the hybrid plants	Reduced establishment or yield of desirable agricultural crops or Increased reservoir for pests and pathogens	No	• Hybrids between the GMOs and other canola would be generated at low levels. • Multiple-herbicide tolerant hybrid canola can be controlled using integrated weed management.
7	Introduced gene for herbicide tolerance	Commercial cultivation of GM canola in agricultural areas ⬇ Cross-pollination with sexually compatible Brassica crops or agricultural weeds ⬇ Establishment of hybrid GM Brassica plants expressing the herbicide tolerance gene as volunteers or Introgression of the introduced herbicide tolerance gene into agricultural weed populations ⬇ Reduced effectiveness of weed management measures to control hybrid volunteers or weeds expressing the herbicide tolerance gene	Reduced establishment or yield of desirable agricultural crops	No	• Hybridisation between GM canola and Brassica crop species would occur at very low levels. • Hybrids between GM canola and Brassica crop species could be controlled by integrated weed management. • Studies have shown it is highly unlikely that the GM herbicide tolerance gene would introgress into Brassicaceae weed species.

2.4.1 Risk scenario 1

Risk source	Introduced gene for herbicide tolerance
Causal pathway	⬇ Commercial cultivation of GM canola expressing the herbicide tolerance gene ⬇ Exposure of people to the introduced protein and resultant metabolites by consumption of oil from GM canola, inhalation of GM canola pollen, or occupational contact with GM canola plants or products ⬇
Potential harm	Increased toxicity or allergenicity for people

Risk source

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

Causal pathway

102. The applicant proposes that Optimum™ GLY Canola would be cultivated on a commercial scale in the canola growing areas of Australia. The GAT4621 herbicide tolerance protein is expected to be present in all GM canola plant parts and at all developmental stages (see Chapter 1, Section 5.5.2). Acetylated amino acids (NAGlu, NAAsp, NAThr, NASer and NAGly), which are produced at higher levels due to GAT4621 enzyme activity, would also be present in all plant parts. Glyphosate metabolites (N-acetyl glyphosate and small quantities of N-acetyl AMPA and AMPA) would be present if the GM canola had been treated with glyphosate herbicide.

103. The general public could be exposed to oil from the GM canola, which would be sold for human consumption. However, processed canola oil does not contain detectable levels of protein, acetylated amino acids or glyphosate metabolites (FSANZ 2014).

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

105. 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.

Potential harm

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

107. The GAT4621 protein is well characterised. Based on all available information, the protein is not known to be toxic or allergenic and does not share relevant sequence homology with known toxins or allergens (Chapter 1, Section 5.2.2), nor is it involved in biochemical pathways that produce toxic or allergenic products (Chapter 1, Section 5.2.3).

108. FSANZ has approved the use of food derived from Optimum™ GLY Canola for human consumption in Australia (Chapter 1, Section 7.2). Food use of Optimum™ GLY Canola has also been approved in other countries including Canada, Japan, Korea, Mexico and the United States (Chapter 1, Section 7.3).

Conclusion

109. Risk scenario 1 is not identified as a substantive risk because the GAT4621 protein and resultant metabolites are not considered toxic or allergenic to humans, the GAT4621 protein and resultant metabolites are not present at detectable levels in GM canola oil, and FSANZ has approved food derived from the GM canola as safe for human consumption. 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 herbicide tolerance
Causal pathway	🠗 Commercial cultivation of GM canola expressing the herbicide tolerance gene 🠗 Exposure of desirable organisms to the introduced protein and resultant metabolites through consumption of GM canola plants or products or contact with GM canola plants 🠗
Potential harm	Increased toxicity for desirable organisms

Risk source

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

Causal pathway

111. The applicant proposes that Optimum™ GLY Canola would be cultivated on a commercial scale and used in the same ways as non-GM canola. It is expected that GM canola seed meal would be routinely used as feed for livestock. Occasionally, whole seeds could also be used as animal feed (OGTR 2011). GM canola could be grazed by livestock over winter if grown as a dual-purpose forage and grain crop, or a failed grain crop could be grazed or cut for hay or silage, or stubble could be grazed after harvest (GRDC 2009). Therefore, livestock would be exposed to the GAT4621 protein and resultant metabolites.

112. Wild animals and birds could enter canola fields and feed on GM canola seed or other plant parts. Pollinators such as honeybees would be exposed to nectar and 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 GAT4621 protein and resultant metabolites.

Potential harm

113. As discussed in Chapter 1, Section 5.2, no acute oral toxicity was observed in a study where purified GAT4621 was fed to mice (Rood 2007). A variety of *in vivo* and *in vitro* toxicity studies were conducted on five acetylated amino acids, which are produced at higher levels in the presence of GAT4621, and found that the acetylated amino acids had no higher toxicity than non-acetylated amino acids (Larson et al. 2011). The metabolites produced by the action of GAT4621 on glyphosate are also expected to have very low toxicity, however, the potential toxicity of herbicide metabolites for animals will be assessed by APVMA when setting the rate at which glyphosate may be applied to Optimum™ GLY Canola.

114. As discussed in Chapter 1, Section 5.5.3, a compositional analysis of Optimum™ GLY Canola seed found no biologically significant differences in the levels of key constituents, including natural toxicants, compared with non-GM canola. Feeding studies in rats and chickens found no significant differences between groups fed on Optimum™ GLY Canola seed products or equivalent products from non-GM canola (Delaney et al. 2014; McNaughton et al. 2014).

115. GAT4621 concentrations in whole plants are similar to the concentration in seeds, which is only 0.002% of total seed protein (Chapter 1, Section 5.5.2). As GAT4621 does not cause toxicity in seeds, it is not expected to lead to toxicity in other plant parts.

116. Based on the findings above, it is not expected that Optimum™ GLY Canola would have increased toxicity towards mammals or birds, whether domesticated or wild. As discussed in Chapter 1, Section 7.3, Optimum™ GLY Canola has been approved for use in animal feed by the relevant regulatory authorities in Canada, Japan, Mexico, Korea and the United States.

117. No studies have tested the potential toxicity of GAT4621 and resultant metabolites towards arthropods or soil organisms. However, the introduced *gat4621* gene was derived from a naturally occurring soil bacterium that is widespread and prevalent in the environment. Therefore, desirable organisms are already exposed to proteins similar to the protein encoded by this gene.

Conclusion

118. Risk Scenario 2 is not identified as a substantive risk because the GAT4621 protein and resultant metabolites are not considered toxic to mammals or birds, the GM canola seed is compositionally equivalent to non-GM canola seed, and proteins similar to GAT4621 are widespread in the environment. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk Scenario 3

Risk source	Introduced gene for herbicide tolerance
Causal pathway	⬇ Commercial cultivation of GM canola in agricultural areas ⬇ Establishment of volunteer GM canola plants expressing the herbicide tolerance gene in agricultural areas ⬇ Reduced effectiveness of weed management measures to control the volunteers ⬇
Potential harm	Reduced establishment or yield of desirable agricultural crops or Increased reservoir for pests and pathogens

Risk source

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

Causal pathway

120. The applicant proposes that Optimum™ GLY Canola would be cultivated on a commercial scale. In current Australian agriculture, canola volunteers requiring weed management are likely to be found in fields for up to three years after growing a canola crop (Australian Oilseeds Federation 2014). As Optimum™ GLY Canola is similar to non-GM canola with respect to the intrinsic characteristics contributing to spread and persistence, such as seed production, shattering, dormancy, and competitiveness (Chapter 1, Section 5.5.3), it would be expected to produce similar numbers of volunteers.

121. Volunteer canola plants are also likely to occur following dispersal of GM canola seeds within agricultural areas. Short-range dispersal of canola seed into field margins or adjacent fields could occur due to pod shattering or transport of canola plant material from windrows by strong winds. 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 or movement of livestock after grazing on canola (OGTR 2011). Dispersal of viable canola seed by endozoochory (consumption and excretion of seed) by wild mammals or birds is also possible at very low levels (Twigg et al. 2008).

122. Glyphosate herbicide is commonly used in broad-acre cropping as a knockdown herbicide for pre-emergent weed control. Glyphosate would not be effective in controlling Optimum™ GLY Canola volunteers due to expression of the introduced herbicide tolerance gene. However, farmers who had planted Optimum™ GLY Canola would be aware of this characteristic, and the Crop Management Plan provided by Pioneer would include information on management strategies for control of GM canola volunteers.

123. All herbicides sold in Australia are grouped by mode of action for the purpose of resistance management. The mode of action is indicated by a letter code on the product label (CropLife Australia 2011). Optimum™ GLY Canola has a GM trait of tolerance to glyphosate, which is a Group M herbicide, and a non-GM trait of tolerance to imidazolinones, which are Group B herbicides. Herbicides from different mode of action groups or products with multiple mode of action groups could be used to control Optimum™ GLY Canola volunteers. Specifically, herbicides from Groups C, F, G, I, L, N and Q are options for use on canola volunteers in various cropping and non-cropping situations. A number of herbicides with multiple mode of action groups (*e.g.* Groups C + F, C + H, C + I, F + I, H + I and L + Q) are also canola volunteer control options (Australian Oilseeds Federation 2014).

124. Thus, GM canola volunteers could be controlled by integrated weed management practices, which would include using a variety of other herbicides approved by the APVMA for use on canola volunteers, as well as non-chemical management methods currently used to control non-GM canola.

Potential harm

125. Volunteer canola is a weed of agricultural production systems (Groves et al. 2003; Simard et al. 2002). If left uncontrolled, volunteer canola plants could reduce the establishment or yield of desired crops. However, GM canola volunteers that are effectively controlled would not be expected to cause greater harm to desired crops than non-GM canola volunteers that are effectively controlled.

126. Volunteer canola could act as a reservoir for canola pests, pathogens or diseases. For example, blackleg is the most serious disease of canola in Australia, and over 95% of blackleg spores originate from the previous year's canola stubble (GRDC 2009). Canola volunteers emerging in fields or field margins the year after a canola crop could be infected with blackleg from stubble, then in turn infect a canola crop planted in the following year. However, there is no difference in disease incidence between Optimum™ GLY Canola and non-GM canola (Chapter 1, Section 5.5.3). GM canola volunteers that are effectively controlled would not be expected to cause greater harm as a disease reservoir than non-GM canola volunteers that are effectively controlled.

Conclusion

127. Risk scenario 3 is not identified as a substantive risk because integrated weed management practices would control GM canola volunteers in agricultural areas. 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 herbicide tolerance
Causal pathway	⬇ Commercial cultivation of GM canola in agricultural areas ⬇ Dispersal of GM canola seed to intensive use areas ⬇ Establishment of feral GM canola plants expressing the herbicide tolerance gene in intensive use areas ⬇ Reduced effectiveness of weed management measures to control the feral plants ⬇
Potential harm	Reduced services from the land use or Reduced biodiversity

Risk source

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

Causal pathway

129. The applicant proposes that Optimum™ GLY Canola would be cultivated for commercial purposes. After harvest, the GM canola seed would usually be transported for processing or export. Seed spillages could lead to establishment of feral canola populations along transport routes or near processing or storage sites. Occasionally whole seeds could be used as livestock feed and feral GM canola could potentially establish in animal feeding areas.

130. As discussed in Chapter 1, Section 4.3, feral canola plants are often observed growing on roadsides or railway easements in Australia. These canola populations are thought to rely on re-supply of seed from spillages rather than forming self-sustaining weed populations. Optimum™ GLY Canola is similar to non-GM canola with respect to the intrinsic characteristics contributing to spread and persistence, such as seed production, shattering, dormancy, and competitiveness (Chapter 1, Section 5.5.3). The genetic modification is also not expected to alter the tolerance of GM plants to biotic or abiotic stresses that normally restrict the geographic range and persistence of canola (Chapter 1, Sections 6.2 and 6.3). Therefore, feral Optimum™ GLY Canola would not be expected to be more persistent than non-GM canola.

131. Glyphosate is widely used for weed control in intensive use areas such as roadsides (Storrie 2012). Glyphosate would not be effective in controlling feral Optimum™ GLY Canola populations due to expression of the introduced herbicide tolerance gene. Broad application of glyphosate in intensive use areas could potentially promote the establishment of feral GM canola due to reduction of competition. A recent Australian study found that under favourable climatic conditions, and in circumstances where other roadside weeds are controlled by glyphosate, roadside populations of glyphosate tolerant GM canola could persist for at least three years (Busi & Powles 2016).

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

(Australian Glyphosate Sustainability Working Group website). These strategies would also be effective in controlling feral Optimum™ GLY Canola.

Potential harm

133. Feral canola on roadsides or along railway lines could potentially reduce services from the land use by obstructing lines of sight around corners and signs, as canola can grow to a height of 1.5 m (OGTR 2011). Also, the Western Australian Department of Parks and Wildlife lists feral canola as one of 60 weeds that threaten rail and roadside vegetation by lowering the biodiversity and aesthetic value of the verge, and recommends that management of these weeds be a priority along roads of high conservation value (Roadside Conservation Committee 2014).

134. A survey of 61 local councils and 25 road and rail authorities in canola growing regions of Australia found that approximately 30% of land managers identified feral canola as a weed present in their area, but approximately 70% of these land managers did nothing to control canola (Dignam 2001), indicating that feral canola was not an issue of high priority. This survey was conducted prior to introduction of GM canola, so glyphosate resistance in canola was not a factor in weed management decisions.

Conclusion

135. Risk scenario 4 is not identified as a substantive risk because canola is not a persistent weed in intensive use areas, weed management strategies other than glyphosate use can control feral GM canola, and most land managers of intensive use areas where feral canola is present do not consider it necessary to control canola. 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 herbicide tolerance
Causal pathway	⬇ Commercial cultivation of GM canola in agricultural areas ⬇ Dispersal of GM canola seed to nature reserves ⬇ Establishment of feral GM canola plants expressing the herbicide tolerance gene in nature reserves ⬇ Reduced effectiveness of weed management measures to control the feral plants ⬇
Potential harm	Reduced establishment of desirable native vegetation

Risk source

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

Causal pathway

137. The applicant proposes that Optimum™ GLY Canola would be cultivated on a commercial scale. If GM canola fields were adjacent to nature reserves, short-range dispersal of canola seed into the nature reserves could occur due to pod shattering or transport of canola plant material from windrows by strong winds, as discussed in Risk Scenario 3. If transport routes for harvested GM canola seeds passed through nature reserves, dispersal of canola seeds into the nature reserves could occur due to spillages, as discussed in Risk Scenario 4. However, surveys of roadside canola typically only found feral canola plants within 5 m of the edge of the road (Agrisearch 2001; Norton 2003). Dispersal of viable canola seed into

nature reserves by endozoochory (consumption and excretion of seed) by wild animals or birds is also possible at very low levels (Twigg et al. 2008).

138. As discussed in Chapter 1, Section 4.1, canola is not considered a significant weed in natural undisturbed habitats in Australia (Dignam 2001; Groves et al. 2003). Optimum™ GLY Canola is similar to non-GM canola with respect to the intrinsic characteristics contributing to spread and persistence, such as seed production, shattering, dormancy, and competitiveness (Chapter 1, Section 5.5.3). The genetic modification is also not expected to alter the tolerance of GM plants to biotic or abiotic stresses that normally restrict the geographic range and persistence of canola (Chapter 1, Sections 6.2 and 6.3). Therefore, Optimum™ GLY Canola would not be expected to be more invasive of natural habitats than non-GM canola.

139. A survey of herbicide usage in 17 national parks found that glyphosate was used as a weed control strategy in less than 5% of the area of national parks, and in most cases herbicide application occurred once a year (Dignam 2001). Glyphosate would not be effective in controlling feral Optimum™ GLY Canola populations due to expression of the introduced herbicide tolerance gene. However, glyphosate could also be ineffective in controlling non-GM canola plants, particularly under conditions of moisture stress (Australian Oilseeds Federation 2014). Therefore, feral GM canola plants would rarely have a survival advantage over non-GM canola plants in nature reserves.

140. A recent Australian study found that when glyphosate tolerant GM canola seeds were dispersed into two natural areas, feral canola populations persisted for 0 and 3 years, respectively, prior to extinction (Busi & Powles 2016).

Potential harm

141. If feral Optimum™ GLY Canola populations 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 in species composition.

Conclusion

142. Risk scenario 5 is not identified as a substantive risk because canola is not considered a significant weed in nature reserves, and the introduced gene does not increase the potential weediness of the GM canola. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.6 Risk Scenario 6

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

Risk source

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

Causal pathway

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

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

146. Hybrid seed with the GM trait could disperse within agricultural areas, to intensive use areas, or to nature reserves, by the same mechanisms described in Risk Scenarios 3-5. In addition, if a field that is adjacent to GMOs is planted with an open pollinating canola variety, the farmer may retain seed, including a proportion of hybrid seed, for future planting.

147. Crossing between the GMOs and non-GM, non-herbicide tolerant canola varieties would result in hybrid plants highly similar to the GMO proposed for release. Therefore, the progeny would not be expected to pose any greater risks than Optimum™ GLY Canola. Likewise, crossing between the GMOs and either Clearfield® canola varieties (tolerant to imidazolinone herbicides) or Roundup Ready® canola varieties (tolerant to glyphosate herbicides) would not produce progeny with different traits to Optimum™ GLY Canola.

148. The other two types of herbicide tolerant canola grown in Australia are TT varieties (tolerant to triazine herbicides) and InVigor® canola (tolerant to glufosinate herbicide), although InVigor® canola is currently only grown in breeding trials. In North America, where canola varieties that are tolerant to different herbicides have been grown in close proximity, the production of multiple-herbicide tolerant volunteers has been noted (Beckie et al. 2003; Hall et al. 2000; Knispel et al. 2008; Schafer et al. 2011). If Optimum™ GLY Canola were to cross with TT and InVigor® canola this could result in a canola with tolerance to four herbicides. This has been a possible outcome since the approval of InVigor® canola and Roundup Ready® canola in 2003. Thus, approval of Optimum™ GLY Canola for commercial release would not add a new trait in terms of combinations of herbicide tolerance in canola volunteers.

149. If dual herbicide tolerant Optimum™ GLY Canola were widely grown, this could increase the likelihood of multiple-herbicide tolerant volunteers, particularly by crossing with TT canola, which is over half of the current Australian canola crop (Pacific Seeds website).

150. However, multiple-herbicide tolerant individuals are as susceptible to alternative herbicides as single-herbicide tolerant canola plants or their non-GM counterparts (Beckie et al. 2004). Therefore, if multiple-herbicide tolerant canola plants were to occur, they could be controlled by other herbicides or by non-chemical agricultural practices such as mowing, tilling or grazing. Triazine herbicides are in mode of action Group C, while glufosinate is in Group N. As discussed in Risk Scenario 3, there are a range of other herbicide products available with alternative or multiple modes of action.

Potential harm

151. If left uncontrolled, volunteer canola plants could establish and compete with other crops. Hybrid canola volunteers with herbicide tolerance traits may not be effectively controlled by existing weed management measures, particularly where herbicide tolerance traits acquired by pollen flow were not anticipated. 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, pathogens or diseases, as described in Risk Scenario 3.

152. However, hybrid canola volunteers are expected to be present at very low densities. Small numbers of volunteers would have limited capacity to cause adverse effects.

Conclusion

153. Risk scenario 6 is not identified as a substantive risk because hybrids between the GMOs and other canola would be generated at low levels, and multiple-herbicide tolerant hybrids can be controlled using alternative herbicides or non-chemical weed management practices. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.7 Risk Scenario 7

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

Risk source

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

Causal pathway

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

<u>Interactions with Brassica crop species</u>

156. Pollen flow between the GM canola proposed for release and other Brassica crop species could occur if the Brassica crops were grown in proximity to 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 (OGTR 2011). Brassica forage crops rarely flower due to heavy grazing. *B. juncea* (Indian mustard) crops, which are grown as oilseeds or for condiment mustard, could plausibly cross-pollinate with the GM canola. Cross pollination could also conceivably occur with volunteer populations of Brassica plants.

157. Hybrids between *B. napus* and *B. juncea* have been observed in the field, are fertile, and often have high fitness (Liu et al. 2010). Cross-pollination between *B. napus* and *B. rapa* occurs frequently in the field if plants of the two species are in proximity, and the hybrids are vigorous and fertile, although with reduced pollen viability (Warwick et al. 2003). Hybrids between *B. napus* and *B. oleracea* have been detected at low levels in wild populations (Ford et al. 2006).

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

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

Interactions with Brassicaceae weeds

160. 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 by weed management prior to flowering, if there is synchronous flowering of weeds and the crop, and if the weed species is sexually compatible with *B. napus*.

161. Cross-pollination between *B. napus* and wild radish (*R. raphanistrum*) has been observed in the field at very low levels. The hybrids are smaller than either parent and close to sterile (Darmency et al. 1998; Warwick et al. 2003). Cross-pollination between *B. napus* and Buchan weed (*H. incana*) has been observed in the field at low levels. The hybrids had very low fertility, and by the fifth generation of back-crossing the progeny produced no viable seed (Darmency & Fleury 2000). Thus, introgression of the herbicide gene from GM canola into wild radish or Buchan weed populations is highly unlikely. Although GM Roundup Ready® canola has been grown commercially in North America since 1996, and wild radish and Buchan weed are both agricultural weeds in North America, there are no reports of glyphosate tolerant wild radish or Buchan weed populations there (The international survey of herbicide resistant weeds; accessed November 2015).

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

163. In the highly unlikely event that the herbicide tolerance gene was introgressed into populations of wild radish or Buchan weed, which retained the vigour of the recurrent weedy parent, these plants could establish as weeds. The GM weeds would not be controlled by the

application of glyphosate herbicide. However, other weed management practices would be as effective on the GM weeds as they are on the parent non-GM weeds.

Potential harm

Interactions with Brassica crop species

164. Both volunteer canola and other Brassica crop species are weeds of agricultural production systems (Groves et al. 2003). Any hybrids between the GM 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. However, if appropriate weed management is used, GM hybrid volunteers would not cause more harm than hybrids between non-GM canola and other Brassica crop species.

Interactions with Brassicaceae weeds

165. According to the Australian Department of the Environment, wild radish and Buchan weed are both declared weeds in canola growing states and are not easily controlled in agricultural areas (National weeds lists; accessed November 2015). If a GM herbicide tolerance trait was introgressed into populations of these weeds, it would increase the difficulty of weed management, particularly where a herbicide tolerance trait was not anticipated. These GM weeds could impact the agricultural environment by reducing the establishment or yield of desired crops.

166. It should be noted that weeds can evolve herbicide resistance through natural mechanisms due to selective pressure. There are reports of wild radish populations in Australia that have acquired resistance to one or more of five classes of herbicides, including glyphosate[4] (The international survey of herbicide resistant weeds; accessed November 2015). If wild radish did acquire a herbicide tolerance gene from GM canola, it would be no more difficult to control than wild radish that had naturally evolved herbicide resistance.

Conclusion

167. Risk scenario 7 is not identified as a substantive risk because hybridisation between GM canola and Brassica crop species would occur at very low levels, hybrids between GM canola and Brassica crop species could be controlled by integrated weed management, and studies have shown it is highly unlikely that the 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

168. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis[5]. There are several types of uncertainty in risk analysis (Bammer & Smithson 2008; Clark & Brinkley 2001; Hayes 2004). These include:

- uncertainty about facts:
  - knowledge – data gaps, errors, small sample size, use of surrogate data

[4] The reported wild radish populations with glyphosate resistance did not acquire their trait from glyphosate tolerant GM canola. The glyphosate resistant wild radish populations were found in Western Australia in 2010 (Ashworth et al. 2014), and GM canola was first commercially grown in Western Australia in 2010, so there was no opportunity for introgression to have occurred.

[5] 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.

  - 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.

169. 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.

170. Optimum™ GLY Canola was approved by the Regulator for limited and controlled release (field trials) under licence DIR 114. The RARMP for DIR 114 identified additional information that could be required to assess a large scale or commercial release of the GM canola. This included additional biochemical characterisation of the GM canola line, and additional phenotypic characterisation of the GM canola line, particularly with respect to traits that may contribute to biotic or abiotic stress tolerance, weediness or persistence. Information provided by the applicant to address these areas of uncertainty is presented and discussed in Chapter 1, Section 5.2 (biochemical characterisation of metabolites) and Chapter 1, Section 5.5.3 (phenotypic and agronomic characterisation).

171. Uncertainty can arise from a lack of experience with the GMO. Optimum™ GLY Canola has not yet been grown commercially anywhere in the world. However, the level of uncertainty is considered to be low given that extensive field trials have been conducted in the United States, Canada and Australia. The uncertainty has been taken into account in assessment of risk scenarios, and is not sufficient to affect the conclusions on the overall level of risk.

172. For commercial releases of GMOs, which typically do not have limited duration, uncertainty regarding any future changes to knowledge about the GMO is addressed through post release review (Chapter 3, Section 4).

Section 4 Risk evaluation

173. 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.

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

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

175. Seven 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 4.

176. 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. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.

Chapter 3 Risk management plan

Section 1 Background

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

178. 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.

179. 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 allow the Regulator, or a person authorised by the Regulator, to enter premises 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.

180. 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

181. 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 Optimum™ GLY Canola. These risk scenarios were considered in the context of the large 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

182. 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

183. 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 (both individuals and the body corporate)
- 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.

184. On the basis of information submitted by the applicant and records held by the OGTR, the Regulator considers Pioneer suitable to hold a licence.

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

186. 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

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

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

188. Any person, including the licence holder, may conduct any permitted dealing with the GMOs.

3.4 Reporting requirements

189. The licence obliges the licence holder to immediately report any of the following to the Regulator:

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

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

191. 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

192. 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.

193. 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

194. Regulation 10 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.

195. For the current application for a DIR licence, the Regulator has incorporated a requirement in the licence for ongoing oversight to provide feedback on the findings of the RARMP and ensure the outcomes remain valid for future findings or changes in circumstances. This ongoing oversight will 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).

196. 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

197. 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), fax (02 6271 4202), 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

198. 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.

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

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

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

202. 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

203. 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 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

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

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

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Appendix A Summary of submissions on RARMP preparation from prescribed experts, agencies and authorities

Before commencing preparation of the RARMP, the Regulator requested submissions from prescribed experts, agencies and authorities on matters considered relevant to the preparation of the RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. The issues raised, and how they are addressed in the consultation RARMP, are summarised below.

Submission	Summary of issues raised	Comment
1	The lead authority would be the Western Australia Department of Agriculture and Food so at this point Shire officers have no further comments on this topic.	Noted. Advice was received from the Western Australia Gene Technology Interdepartmental Committee, including representatives from the Department of Agriculture and Food.
2	The RARMP should consider: • 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 desirable organisms through toxicity • whether the introduced herbicide tolerance trait will increase the potential for the GM canola to spread and persist, leading to harm to the environment • the potential for gene flow to other canola, including other GM or non-GM herbicide tolerant canola, and whether this could lead to harm to the environment. • the potential for the introduced glyphosate acetyltransferase protein to acetylate non protein amino acids, in the context of their role in the food chain	The potential for the GM canola to be toxic or allergenic to people is addressed in Risk Scenario 1 in Chapter 2 of the RARMP. The potential for the GM canola to be toxic to other desirable organisms is addressed in Risk Scenario 2 in Chapter 2 of the RARMP. The potential for the herbicide tolerance trait to increase spread and persistence of the GM canola, leading to harms to the environment in different land uses, is addressed in Risk Scenarios 3-5 in Chapter 2 of the RARMP. The potential for hybridisation with other canola is addressed in Risk Scenario 6 in Chapter 2 of the RARMP. The potential for acetylation of non-protein amino acids, and possible resultant toxicity, is discussed in Section 5.2.3 in Chapter 1 of the RARMP. The consultation RARMP for this release concludes that risks to human health and the environment are negligible.
3	Does not support the proposed field trial of GM canola in the local government area. States that without access to unbiased, expert data, scientific knowledge and a clear directive from the State Government, a precautionary approach to any such trial must be taken to reduce the risk of potential environmental and economic damage to the community and surrounding local government areas. Not yet convinced that the release of GM products without significant direct benefits to public health should be permitted. The region includes a Shire with the motto of 'pure' which is seen as providing marketing advantages. Growing, storage and transport of GM crops within this area would be in direct opposition to this marketing strategy. Mentions media articles highlighting the difficulties of treating roadside vegetation that is now immune to easy chemical treatment. It is suggested that this excessive vegetation is often	This application is for a commercial release of GM canola. If the application is approved, the GM canola may be sold to farmers who may grow it anywhere in Australia. The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology. The RARMP informs the Regulator when making a decision whether or not to issue a licence. Each RARMP includes a critical assessment of data supplied by the applicant, together with a review of other relevant national and international scientific literature. The RARMP is finalised following an extensive consultation process, including advice from independent scientific experts and State governments. Marketing and trade issues are outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence. These matters fall under the jurisdiction of individual States and Territories. The potential for feral GM canola to establish in roadsides and railway easements is addressed in Risk Scenario 4 in Chapter 2 of the RARMP. It is considered that feral GM

Submission	Summary of issues raised	Comment
	from a GM product. Most roadsides and railway easements in the region now have fugitive canola growing with little or no treatment from the relevant authorities. If the trial is approved would expect safeguards put in place to prevent escape of the GM canola from the trial sites. Considers canola a high risk crop for pollen mediated gene flow, expects trial areas to be well protected to ensure that bees do not spread the GM canola, and suggests that further research is required to resolve the issue of gene flow. The local government area has significant variation in topography, soil, water and other physical attributes. Council would like to be informed of the exact location of the trials in order to provide a more meaningful response from people familiar with the area. Believes it is important that any GM application receive a broad public notification and opportunity for comment so that informed choices can be made by more than just the regulators and the supporters for GM releases.	canola can be controlled by a range of herbicides other than glyphosate, or by non-chemical weed management strategies. The potential for dispersal of GM canola seeds from agricultural fields is addressed in Risk Scenarios 3 – 5 in Chapter 2 of the RARMP. The potential for gene flow from the GM canola to other canola is addressed in Risk Scenario 6 in Chapter 2 of the RARMP. The consultation RARMP for this release concludes that risks to human health and the environment are negligible. In the first round of consultation, comments on this application were invited from prescribed experts, Australian Government authorities and agencies, State and Territory Governments, relevant Australian local councils and the Minister for the Environment. In the second round of consultation, comments on the consultation RARMP are invited from all the stakeholders previously consulted on the application and also from the general public. The public notification includes advertising in the general circulation newspaper The Australian, four rural newspapers, the Australian Government Gazette and on the OGTR website. The invitation to comment is also sent to interested parties who have registered on the OGTR mailing list and a tweet will be broadcast by the Department of Health's Twitter account. All submissions will be considered in finalising the RARMP, which will then inform the Regulator's decision on whether or not to issue a licence.
4	Nil comments.	Noted.
5	Given the biology and ecology of canola, as well as the nature of the introduced trait, considers that the risks posed to the environment by the proposed release are minimal. The RARMP should take into account: • the context of the wide range of herbicide resistant varieties (GM and non-GM) cultivated, or potentially to be cultivated, in Australia, and the environmental impact of these herbicide tolerant plants • the possibility that Optimum™ GLY Canola could hybridise with any commercially available canolas, potentially leading to plants with an ability to tolerate multiple herbicides, which could reduce the options available for the elimination of volunteer plants • routes for dispersal of canola seed to land outside of cultivation; spread and persistence of feral populations of canola in the environment; the adverse impacts of feral canola on the environment; and options for the elimination of feral GM canola • the potential weediness of the GM canola, especially that the trait of herbicide tolerance is expected to only produce any	The different herbicide tolerant canola varieties present in Australia are discussed in Section 6.4 of Chapter 1 of the RARMP. This range of properties in the parent organism is used as context when assessing potential risks to the environment associated with release of the GM canola. The potential for the GM canola to hybridise with other herbicide tolerant canolas, leading to multiple-herbicide tolerant plants and reduced effectiveness of weed management, is addressed in Risk Scenario 6 in Chapter 2 of the RARMP. The potential for dispersal of GM canola to non-agricultural areas, and adverse effects of feral GM canola populations on the environment, are addressed in Risk Scenarios 4 and 5 in Chapter 2 of the RARMP. The potential for weediness of the GM canola in different land uses is addressed in Risk Scenarios 3-5 in Chapter 2 of the RARMP. These risk scenarios consider how glyphosate herbicide may be applied in these land uses and whether the trait of herbicide tolerance would confer any advantage. The potential for cross-pollination between the GM canola and sexually compatible species is addressed in Risk Scenario 7 in Chapter 2 of the RARMP. The consultation RARMP for this release concludes that risks to human health and the environment are negligible.

Submission	Summary of issues raised	Comment
	selective advantage for plants in the environment in situations where the herbicide is applied • the potential for gene flow from the GM canola to sexually compatible species, including Brassica juncea, wild radish, charlock and Buchan weed	
6	Canola seed for sowing is currently permitted entry into Australia subject to general seed for sowing conditions listed on the Import Conditions Database. It is not subject to specific import conditions for specific quarantine pathogens. Considered unlikely that the quarantine risk posed by the GM Canola differs from non-GM canola in terms of pests and diseases that may be associated with the canola seed. However, GM canola bred for herbicide resistance may be more difficult to manage than non-GM canola if it escapes cultivation and becomes a weed of other crops	The potential for the herbicide tolerant GM canola to become a weed of other crops is addressed in Risk Scenario 3 in Chapter 2 of the RARMP. The consultation RARMP for this release concludes that risks to human health and the environment are negligible.
7	States as background that landscapes in the Shire have high biodiversity values and are important to social wellbeing and to the economy as a key element of tourism. Council received a petition indicating community concern regarding the introduction of GMOs, and passed a resolution that Council: • Adopt a precautionary approach to the introduction of GM crops by advocating to State Government to oppose the introduction of GM crops into the Shire, and advocating for the mandatory labelling of all GM products. • This precautionary approach should remain until the following safeguards are in place: mandatory labelling on all products produced using gene technology; GM-free zones have been established where GM-free crops can be grown safely; independent research results showing that specific GMOs are harmless to health and the environment are undertaken; and a strong and enforceable liability and insurance regime is in place for GMO products. Note that Council has written to the State Government advocating that they oppose the introduction of GM crops into the Shire. Council officers have concerns that approval of an unrestricted licence to release GM canola in all commercial canola growing areas in Australia would fail to take into account locally significant risks to health, safety and the environment. Encourage the Regulator to consider working within local areas to determine the appropriateness or otherwise of GM crops.	Some areas may be declared GM free under State or Territory law for marketing purposes. This is a decision that falls under the jurisdiction of individual State or Territory governments. Marketing and trade issues, including segregation and coexistence regimes, are outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence. FSANZ has regulatory responsibility for food safety assessment and labelling, including for GM food. Product labelling is outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence. The Act requires the Regulator to identify and manage risks to human health and safety and the environment posed by or as a result of gene technology. The RARMP informs the Regulator when making a decision whether or not to issue a licence. Each RARMP includes a critical assessment of data supplied by the applicant, together with a review of other relevant national and international scientific literature. The RARMP is finalised following an extensive consultation process, including advice from independent scientific experts. The consultation RARMP for this release concludes that risks to human health and the environment are negligible. Because the consultation RARMP finds that the GM canola poses no greater risks to human health and the environment than non-GM canola, the draft licence in Chapter 4 of the RARMP does not require the licence holder to be insured against any harm that may be caused by the GMOs. In the unlikely event that GM products were to cause harm, questions of liability would fall under common law. For an application for a commercial licence, the Act requires two rounds of consultation with relevant local government areas, and one round of consultation with the general public. The public invitation to comment for this application includes advertising in the general circulation newspaper The Australian and four rural newspapers

Submission	Summary of issues raised	Comment
		published in Australian canola growing areas. Any issues raised relating to human health and safety and the environment that may be specific to local areas are taken into account when finalising the RARMP.
8	Raises no issues to be considered in the preparation of the RARMP.	Noted.
9	Have no problems with this application.	Noted.
10	The Regulator should consider the comments below during preparation of the RARMP: • although the applicant provides details of a qPCR assay to detect the GMOs in a laboratory, there is no mention of development of a rapid in-field test • the applicant proposes to monitor compliance with its stewardship program, but does not specify if the data collected would be provided to the OGTR • the parent organism is present in the environment as a non-persistent weed, not only on roadsides • a single trait may lead to weediness	A draft licence condition in Chapter 4 of the RARMP requires the licence holder to provide the Regulator with a methodology to reliably detect the GMOs or the presence of the genetic modifications in a recipient organism. A laboratory test that could be used on samples taken from the field would meet this requirement. A draft licence condition in Chapter 4 of the RARMP requires the licence holder to inform the Regulator of any additional information as to risks to human health or the environment associated with the GMOs, or any unintended effects of the GMOs, or any contraventions of the licence conditions. However the licence holder becomes aware of this information, the Regulator must be informed without delay. Section 4.3 of Chapter 1 of the RARMP discusses the presence of the parent organism as a weed in the environment in a range of land uses. Risk Scenarios 3-5 in Chapter 2 of the RARMP address the potential for the introduced trait of herbicide tolerance to lead to weediness of the GM canola in different land uses. The consultation RARMP for this release concludes that risks to human health and the environment are negligible.

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

The Regulator received a number of submissions from prescribed experts, agencies and authorities[6] 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	Does not have an official position on GM foods or crops. Would want the assessments to indicate there is no serious public health and safety or environmental risks from growing modified canola. Similarly from the consumption of products with GM canola. The issue of public being able to identify what has GM in their food products should be dealt with by other agencies appropriately so that consumers can made an informed choice about purchasing foods with such ingredients.	The risk assessment concludes that the commercial release of this GM canola poses negligible risks to the health and safety of people or to the environment. FSANZ has regulatory responsibility for food safety assessments and food labelling in Australia, including GM food. FSANZ has assessed and approved food derived from the GM canola as safe for human consumption. Labelling of GM status is legally required for GM foods that contain novel DNA or protein or have altered characteristics.
2	Provides an information sheet that outlines concerns that have been raised about human exposure to the herbicide glyphosate, describes how the APVMA is reviewing these concerns, and advises Councils to continue using glyphosate products according to label instructions until the review process is complete. Queries whether the use of this herbicide is going to continue in light of the gene modifications occurring.	The GM canola is designed to be tolerant to the herbicide glyphosate, with the intention of applying glyphosate to control weeds in the crop. Pioneer has submitted an application to APVMA to use glyphosate with the GM canola. The APVMA has regulatory responsibility for herbicide use in Australia.
3	Notes that Section 4 of South Australia's *Genetically Modified Crops Management Regulations 2008* states that "the whole of the State is designated as an area in which no genetically modified food crops may be cultivated". In 2012, Council adopted a *Genetically Modified Crops* policy which, in essence, states that "Council does not support the growing of genetically modified crops within its District". This policy was adopted after a public consultation process. In considering the submissions, Council determined that there is an absence of credible and independent scientific evidence that GM crops, either generally or specifically, are safe for people or the environment. Until such evidence can be presented to Council, this policy will remain in effect. In this regard, Council is concerned that the Office of the Gene Technology Regulator has chosen to consider "risks" associated with product release, rather than seek assurances of the product's safety for humans and its release environment. In saying this, Council is aware of the considerable pressures of the	State or Territory governments can declare areas to be GM-free for marketing purposes. The licence does not authorise dealings with GM canola that are otherwise prohibited as a result of the operation of State legislation. The RARMP concludes that the commercial release of this GM canola poses negligible risks to the health and safety of people or the environment. The RARMP was prepared using a combination of critical assessment of data provided by the applicant, review of published scientific literature, information on relevant previous approvals and any adverse effects of these releases, and advice received from a range of Australian government authorities, agencies, experts and the public. The *Gene Technology Act 2000* requires the Gene Technology Regulator to prepare a risk assessment for licence applications, which takes into account risks to the health and safety of people or risks to the environment. Therefore, the Regulator's assessment must be framed in terms of risks

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

Submission	Summary of issues raised	Comment
	commercial context in which the issue of the risks of genetically modified organisms is being assessed. Council urges the Regulator to withhold permission for the commercial use of any genetically modified crops until their safety has been credibly and independently demonstrated, rather than their risk deemed acceptable.	associated with product release. Note that risk assessments and safety assessments consider the same issues.
4	Notes that the RARMP states that levels of acetylated amino acids are much higher in the GM seed than in the control seed, and asks for quantitative data to be provided in the RARMP. Agrees with the overall conclusion of the RARMP and raises no further issues.	A table showing concentrations of acetylated amino acids in GM and non-GM canola seeds has been added to Section 5.2.1 of Chapter 1 of the RARMP.
5	Council does not have the specialist scientific expertise available to comment in any detail on the risks of a trial of GM crops in the region but it is important to highlight that a Council resolution was passed in 2001 declaring the municipality to be a GM free district. The shire is committed to having a clean and green image that is demonstrated by the significant number of organic famers that operate in the region. Council keeps a list of these farms in order to ensure that they are notified if Council is carrying out any weed control works that involve spraying of chemicals near their property. This ensures that chemical spraying is undertaken very carefully in these areas so that there is no impact on the farmer's organic accreditation. It is impossible for Council to give any useful feedback on local issues without more detail on the location of the trial site. If Council had more detail on the location of the site then it could try to highlight local issues that would need to be managed such as the issues associated with the location of organic farms.	There is no specific trial site location under this licence, as the licence authorises a commercial release. GM canola seed may be sold to farmers and grown anywhere in Australia. Marketing and trade issues, including segregation and coexistence regimes, are outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence. These matters fall under the jurisdiction of individual States and Territories. State or Territory governments can declare areas to be GM-free for marketing purposes.
6	Satisfied with the conclusions of the draft RARMP and has no further comments.	Noted.
7	Raises no issues to be considered in the preparation of the RARMP.	Noted.
8	Suggests that the paper *Transgenic glyphosate-resistant canola can persist outside agricultural fields in Australia*, published in 2016, could be included as a reference in risk scenarios in the RARMP. Overall, supports the Office of the Gene Technology Regulator's conclusion that DIR 139 poses negligible risk of harm to human health and safety and the environment.	Relevant data from this recent paper has been added to the RARMP. The paper is cited in Risk Scenarios 4 and 5 in Chapter 2 of the RARMP.
9	Supportive of the application as the consultation RARMP indicates that the proposed release poses negligible risks to people or the environment. It is understood that a range of licence conditions would ensure there is ongoing oversight of the release. It is also noted that food made from this genetically modified canola has been assessed and approved by FSANZ as safe for human consumption.	Noted.
10	A variety of GM canola with different genes for glyphosate tolerance has been commercially grown in Australia since 2008. Notes that the introduced protein	Noted.

Submission	Summary of issues raised	Comment
	has a history of safe use in the food industry and food from this GM canola has been assessed as safe for human consumption by FSANZ. Also notes that licence conditions for this application stipulate that a risk management plan must be used in its commercial cultivation. For these reasons, concurs with the Acting Regulator's assessment that under the licence conditions proposed, the dealing poses negligible risks to the health and safety of persons and the environment.	

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

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

Summary of issues raised	Comment
Objects to this release. Increased use of glyphosate is undesirable for health and sustainability reasons.	Issues relating to herbicide use are outside the scope of the Regulator's assessments. The APVMA has regulatory responsibility for agricultural chemicals, including herbicides, in Australia. The APVMA considers risks to human health and the environment in assessing agricultural chemicals for registration and in setting maximum application rates. Further information on the safety of glyphosate is available on the APVMA website.
Human and environmental health and safety are dependent upon naturally functioning ecosystems. Techniques that offer excessive disruption and simplification of these systems should be avoided. Species extinction and climate change warn us to stop human impact and restore natural systems.	Noted. The RARMP found that cultivation of the GM canola poses no greater risks to human health and the environment than cultivation of commercial non GM canola.