

Australian Government

Department of Health and Ageing Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 108

Commercial release of canola genetically modified for herbicide tolerance and a hybrid breeding system (InVigor® x Roundup Ready® canola)

Applicant: Bayer CropScience Pty Ltd

December 2011

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Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of application DIR 108 from Bayer CropScience Pty Ltd (Bayer). The licence authorises dealings involving the commercial release of genetically modified (GM) canola into the environment.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether or not to issue a licence to deal with a genetically modified organism (GMO).

The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with requirements of the legislation. RARMPs apply the *Risk Analysis Framework* and are finalised following consultation with a wide range of experts, agencies and authorities, and the public¹.

The application

Bayer has applied for a licence for dealings involving the intentional release of GM InVigor® x Roundup Ready® canola. Bayer is seeking approval to release the GM canola in all commercial canola growing areas of Australia. The GM canola and products derived from the GM canola would enter general commerce, including use in human food and animal feed.

Note that cultivation of GM canola may also be subject to other requirements in some Australian States and Territories for marketing reasons.

GM InVigor® x Roundup Ready® canola was produced by conventional breeding between GM InVigor® canola and GM Roundup Ready® canola, which were individually approved by the Regulator in 2003 for commercial release under licences DIR 021/2002 and DIR 020/2002, respectively.

The GM InVigor® x Roundup Ready® canola proposed for commercial release contains genes from common bacteria conferring tolerance to the herbicides glufosinate ammonium and glyphosate. In addition, some of the GM canolas proposed for release contain genes from common bacteria conferring a hybrid breeding system and/or an antibiotic resistance gene. The antibiotic resistance gene, which confers tolerance to the antibiotic kanamycin, was used to select genetically modified plants during their initial development in the laboratory.

GM InVigor® x Roundup Ready® canola has been previously approved for field trials in Australia under licences DIR 069/2006 and DIR 104 issued to Bayer.

Food Standards Australia New Zealand (FSANZ) has approved the use of food derived from GM InVigor® canola and GM Roundup Ready® canola for human consumption. These approvals also cover GM InVigor® x Roundup Ready® canola.

The Australian Pesticides and Veterinary Medicines Authority (APVMA) has regulatory responsibility for the supply of agricultural chemicals, including herbicides, in Australia. Amendments to the labels of glufosinate ammonium and glyphosate herbicides would be

¹ More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the <u>Office of the Gene Technology Regulator</u> (OGTR) (Free call 1800 181 030 and in the <u>Regulator's *Risk Analysis Framework*</u> (OGTR 2009).

required for them to be used on commercial scale plantings of InVigor® x Roundup Ready® canola.

An Australian Quarantine and Inspection Service (AQIS) permit would be required to allow the importation of seed.

Risk assessment

The risk assessment took into account information in the application, previous approvals, relevant scientific/technical knowledge and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP. Advice relating to risks to human health and safety and the environment provided in submissions received during consultation on the RARMP has also been considered. No new risks to people or the environment were identified from the advice received on the consultation RARMP.

Initially, potential pathways that might lead to harm to people or the environment as a result of gene technology are postulated (risk scenarios), and those that warrant detailed characterisation are determined. This process is described as risk identification.

Five risk scenarios were postulated, including consideration of whether or not expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms, or alter characteristics that may impact on the spread and persistence of the GM canola. The opportunity for gene flow to other organisms, and its effects if it were to occur, were also assessed.

A **risk** is only identified for further assessment when a risk scenario is considered to have some chance of causing harm. Pathways that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

The characterisation of the five risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the large scale of the release proposed by the applicant and considering both the short and long term, did not identify any risks that could be greater than negligible. Therefore, they did not warrant further detailed assessment.

Risks to the health and safety of people, or the environment, from the proposed release of GM canola into the environment are assessed to be **negligible**.

Risk management plan

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

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. As the risks to the health and safety of people or the environment from the proposed dealings are assessed to be **negligible**, no specific risk treatment measures are imposed.

However, the Regulator has imposed licence conditions under post-release review (PRR) to ensure that there is ongoing oversight of the release and to allow the collection of information to verify the findings of the RARMP.

The licence also contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements, which include an obligation to report any unintended effects.

Conclusions of the RARMP

The risk assessment concluded that this commercial release of GM InVigor® x Roundup Ready® canola to be grown throughout Australia, and the entry of products derived from the GM canola into general commerce Australia-wide, poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded 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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Abbreviations

the Act	Gene Technology Act 2000
APHIS	Animal and Plant Health Inspection Service of the US Department
	of Agriculture
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
CaMV	Cauliflower mosaic virus
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
ELISA	Enzyme linked immunosorbent assay
EPA	(United States) Environmental Protection Agency
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
FDA	(United States) Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
FMV	Figwort mosaic virus
GM	Genetically Modified
GMAC	Genetic Manipulation Advisory Committee
GMO	Genetically Modified Organism
GOX	glyphosate oxidoreductase
HGT	Horizontal gene transfer
kD	kilodalton
LGA	Local Government Area
m	metre
mm	millimetre
mRNA	messenger ribonucleic acid
MS	Male-sterile
nptII	neomycin phosphotransferase II gene
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
PAT	phosphinothricin acetyl transferase
PRR	Post release review
RARMP	Risk Assessment and Management Plan
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
RF	Restorer of fertility
RNA	Ribonucleic Acid
T-DNA	Transfer DNA
Ti	Tumour inducing
TT	Triazine tolerant
USDA	United States Department of Agriculture

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Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of application DIR 108 from Bayer CropScience Pty Ltd (Bayer). The licence authorises dealings involving the commercial release of genetically modified (GM) canola into the environment.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Gene Technology Regulator (the Regulator) before making a decision whether to issue a licence to deal with a genetically modified organism (GMO).

The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with requirements of the legislation. RARMPs apply the *Risk Analysis Framework* and are finalised following consultation with a wide range of experts, agencies and authorities, and the public².

The application

Bayer has applied for a licence for dealings involving the intentional release of GM InVigor® x Roundup Ready® canola. Bayer is seeking approval to release the GM canola in all commercial canola growing areas of Australia. The GM canola and products derived from the GM canola would enter general commerce, including use in human food and animal feed.

Note that cultivation of GM canola may also be subject to other requirements in some Australian States and Territories for marketing reasons.

GM InVigor® x Roundup Ready® canola was produced by conventional breeding between GM InVigor® canola and GM Roundup Ready® canola, which were individually approved by the Regulator in 2003 for commercial release under licences DIR 021/2002 and DIR 020/2002, respectively.

All GM InVigor[®] x Roundup Ready[®] canola proposed for commercial release contains genes conferring tolerance to the herbicides glufosinate ammonium and glyphosate. In addition, some of the GM canolas proposed for release contain genes conferring a hybrid breeding system and/or an antibiotic resistance gene, depending on the specific GM InVigor[®] parent line³.

Glyphosate tolerance is conferred by expression of the *goxv247* gene (a modified version of the *gox* gene obtained from the soil bacterium *Ochrobactrum anthropi* strain LBAA) and the *cp4 epsps* gene obtained from the soil bacterium *Agrobacterium tumefaciens* strain CP4. The *goxv247* gene encodes glyphosate oxidoreductase, an enzyme capable of degrading glyphosate into non-toxic metabolites. The *cp4 epsps* gene encodes a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme. EPSPS enzymes participate in a biosynthetic pathway found in both plants and microorganisms that is required for the synthesis of some essential amino acids. Most plant EPSPS enzymes are inhibited by

² More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the <u>Office of the Gene Technology Regulator</u> (OGTR) (Free call 1800 181 030 and in the <u>Regulator's *Risk Analysis Framework* (</u>OGTR 2009).

³The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

glyphosate, which results in plant death due to the lack of essential amino acids. However, CP4 EPSPS has a much lower affinity for glyphosate than related plant EPSPS enzymes and can continue to function in the presence of glyphosate.

Glufosinate ammonium herbicide tolerance is conferred by expression of the *pat* gene obtained from *Streptomyces viridochromogenes* or the *bar* gene obtained from *Streptomyces hygroscopicus*. Both genes encode functionally equivalent phosphinothricin acetyltransferase enzymes that alter the structure of the active component in glufosinate ammonium herbicides, rendering the herbicide inactive.

Some of the GM canolas proposed for release contain the *barnase* and/or *barstar* genes obtained from the soil bacterium *Bacillus amyloliquefaciens*. *Barnase* encodes a ribonuclease enzyme (BARNASE), and *barstar* encodes a specific inhibitor of the BARNASE enzyme. BARNASE is produced specifically in the anthers of GM flowers and prevents pollen production, resulting in male-sterility. Production of BARSTAR in the same cells inhibits BARNASE activity to restore fertility of the flower.

Some of the GM canolas also contain the *nptll* gene obtained from the bacterium *Escherichia coli*. The *nptll* gene encodes the enzyme neomycin phosphotransferase II which confers resistance to kanamycin and structurally related antibiotics. During development of the GM canola, this marker gene enabled selection of genetically modified plant tissues.

Short regulatory sequences necessary to control expression of the novel genes are present in GM InVigor® x Roundup Ready® canola. These sequences have been derived from: the common soil bacterium *A. tumefaciens*; the plant species *Arabidopsis thaliana* (thale cress), *Nicotiana tabacum* (tobacco) and *Pisum sativum* (pea); and the plant viral pathogens Cauliflower Mosaic Virus and Figwort Mosaic Virus. Although *A. tumefaciens*, Cauliflower Mosaic Virus and Figwort Mosaic Virus are plant pathogens, the regulatory sequences comprise only a small part of their total genome, and are not in themselves capable of causing disease.

GM InVigor® x Roundup Ready® canola has been previously approved for field trials in Australia under licences DIR 069/2006 and DIR 104 issued to Bayer.

Risk assessment

The risk assessment took into account information in the application, previous approvals, relevant scientific/technical knowledge, and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP. Advice relating to risks to human health and safety and the environment provided in submissions received during consultation on the RARMP has also been considered. No new risks to people or the environment were identified from the advice received on the consultation RARMP.

A reference document, *The Biology of* Brassica napus *(canola)*, was produced to inform the risk assessment process for licence applications involving GM canola plants. The document is available from the OGTR or from the website http://www.ogtr.gov.au.

Initially, potential pathways that might lead to harm to people or the environment as a result of gene technology are postulated (risk scenarios), and those that warrant detailed characterisation are determined. This process is described as risk identification.

Five risk scenarios were postulated, including consideration of whether or not expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms, or alter characteristics that may impact on the spread and persistence of the GM

canola. The opportunity for gene flow to other organisms, and its effects if it were to occur, were also assessed.

A **risk** is only identified for further assessment when a risk scenario is considered to have some chance of causing harm. Pathways that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

The characterisation of the five risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the large scale of the release proposed by the applicant and considering both the short and long term, did not identify any risks that could be greater than negligible. Therefore, they did not warrant further detailed assessment. The principal reasons for this include:

- the GM canola has been produced by conventional breeding of GM canola lines that have previously been assessed and authorised for commercial release in Australia
- widespread presence of the same or similar proteins encoded by the introduced genes in the environment and lack of known toxicity or evidence of harm from them
- limited capacity of the GM canola to spread and persist in undisturbed natural habitats.

Risks to the health and safety of people, or the environment, from the proposed release of GM canola into the environment are assessed to be **negligible**.

Risk management plan

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

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. As the risks to the health and safety of people or the environment from the proposed dealings are assessed to be **negligible**, no specific risk treatment measures are imposed.

However, the Regulator has imposed licence conditions under post-release review (PRR) to ensure that there is ongoing oversight of the release and to allow the collection of information to verify the findings of the RARMP.

The licence also contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements, which include an obligation to report any unintended effects.

Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by other agencies that also regulate GMOs or GM products including FSANZ, the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration (TGA), the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and the Australian Quarantine and Inspection Service (AQIS)⁴.

⁴ More information on Australia's integrated regulatory framework for gene technology is contained in <u>the *Risk*</u> <u>Analysis Framework</u> available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030.

FSANZ is responsible for human food safety assessment, including GM food. FSANZ has approved the use of food derived from GM InVigor® canola and GM Roundup Ready® canola for human consumption. These approvals also cover GM InVigor® x Roundup Ready® canola.

APVMA has regulatory responsibility for the supply of agricultural chemicals, including herbicides, in Australia. Amendments to the labels of glufosinate ammonium and glyphosate herbicides would be required for them to be used on commercial scale plantings of InVigor® x Roundup Ready® canola.

An AQIS permit would be required to allow the importation of seed.

In addition, dealings authorised by the Regulator may be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

Suitability of the applicant

The Regulator has assessed the suitability of Bayer CropScience Pty Ltd to hold a DIR licence as required by the Act. Bayer is considered suitable as the Regulator is satisfied that no relevant convictions have been recorded, no licences or permits have been cancelled or suspended under laws relating to the health and safety of people or the environment, and the organisation has the capacity to meet the conditions of the licence.

Conclusions of the consultation RARMP

The risk assessment concluded that this commercial release of GM InVigor® x Roundup Ready® canola to be grown throughout Australia, and the entry of products derived from the GM canola into general commerce Australia-wide, poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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

Chapter 1 Risk assessment context

Section 1 Background

1. 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 (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	PARENT ORGANISM Origin and taxonomy Cultivation and use Biological characterisation Ecology			
Introduced genes (genotype) Novel traits (phenotype) PREVIOUS RELEASES	RECEIVING ENVIRONMENT Environmental conditions Agronomic practices Presence of related species Presence of similar genes			

Figure 1 Parameters used to establish the risk assessment context

2. The risk assessment context is developed within the framework of the *Gene Technology Act 2000* (the Act) and Gene Technology Regulations 2001 (the Regulations, Section 2), the *Risk Analysis Framework*, and operational policies and guidelines available at the OGTR website http://www.ogtr.gov.au

3. In addition, establishing the risk assessment context for this application includes consideration of:

- the proposed dealings (Section 3)
- the parent organism (Section 4), including the genetically modified (GM) parent organisms (Section 5)
- the genetically modified organisms (GMOs), nature and effect of the genetic modification (Section 6)
- the receiving environment (Section 7)
- previous releases of these or other GMOs relevant to this application (Section 8).

Section 2 The legislative requirements

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and with whom he must consult, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of his decisions on licence applications. In addition, the Regulations outline 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. This means that, under section 50(3) of the Act, the Regulator was required to consult with prescribed experts, agencies and authorities to seek advice on matters relevant to the preparation of the RARMP. This first round of consultation included the Gene Technology Technical Advisory Committee, State and Territory Governments, Australian Governments, Australian Government authorities or agencies prescribed in the Regulations, any local council that the Regulator considered appropriate⁵ 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. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix B. Nine submissions were received from the public on the consultation RARMP and the issues raised and their considerations are summarised in Appendix C.

Section 3 The proposed release

3.1 The proposed dealings

7. Bayer CropScience Pty Ltd (Bayer) proposes to release into the environment GM canola that has been genetically modified for herbicide tolerance and a hybrid breeding system. The GM canola proposed for release is the product of conventional breeding between GM canola lines⁶ approved for commercial release under licences DIR 021/2002 (InVigor® canola, including lines MS1, MS8, RF1, RF2, RF3, T45, and Topas 19/2, and hybrids of these) and DIR 020/2002 (Roundup Ready® canola line GT73).

8. The applicant proposes the release to occur in all commercial canola growing areas of Australia. No controls are proposed to restrict the release. GM canola and GM canola-derived products from the GMO would enter general commerce, including use in human food and animal feed.

9. The dealings involved in the proposed intentional release would include:

- conducting experiments with the GMO
- making, developing, producing or manufacturing the GMO
- breeding the GMO with Australian canola cultivars
- propagating the GMO
- using the GMO in the course of manufacture of a thing that is not the GMO
- growing, raising or culturing the GMO
- transporting the GMO
- disposing of the GMO
- importing the GMO
- the possession, supply or use of the GMO for the purposes of, or in the course of, any of the above.

⁵ Bayer is seeking approval for unrestricted commercial release of InVigor® x Roundup Ready® canola in 'all canola cropping areas of Australia'. This involves a significant proportion of the land in the Australian winter cereal belt of NSW, Victoria, South Australia, and Western Australia. It also includes Southern Queensland and Tasmania. Therefore, the Regulator decided to consult with all of the local councils in Australia.

⁶The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

10. Initially, the applicant has proposed to conduct limited demonstration trials and small-scale seed production. After this initial release, seeds would be sold for commercial production in areas that are suitable for growing InVigor® x Roundup Ready® canola.

11. When producing certified InVigor® x Roundup Ready® canola seed, the applicant proposes to use standard certified seed production methods, including maintaining a 400 m isolation distance from other commercial canola crops.

12. Industry has developed guidelines for growing and dealing with GM and non-GM canola to enable the coexistence of GM and non-GM production systems and supply chains (Gene Technology Grains Committee 2003). To comply with these guidelines, Bayer proposes that all resellers of InVigor® x Roundup Ready® canola seed will be trained and accredited, thereby providing all growers with information on the use of the crop, management strategies for control of volunteers, and all industry guideline requirements. Detailed instructions and recommendations for growing InVigor® x Roundup Ready® canola will also be delivered via several other mechanisms, including the seed labels, herbicide labels and the crop management plans for InVigor® canola and Roundup Ready® canola, developed by Bayer and Monsanto respectively.

Section 4 The parent organism

13. The parent organism is *Brassica napus* L. ssp. *oleifera*, which is commonly known as canola, rapeseed or oilseed rape. The GM canola lines that are the parents of the GMOs proposed for release are discussed in Section 5 below.

14. Canola is exotic to Australia and grown as an agricultural crop mainly in New South Wales, Victoria, South Australia and Western Australia. Canola has been grown in Australia since the 1960s primarily for its seeds, which yield from 35% to over 45% oil. Further information about the parent organism is contained in a reference document, *The Biology of* Brassica napus *L. (canola)*, that was produced to inform the risk assessment process for licence applications involving GM canola plants (OGTR 2011).

4.1 Toxicity of non-GM canola

15. Canola seeds are used to produce two major products, canola oil and meal, but only the oil is used in human food. *B. napus* contains two natural toxicants in the seed: erucic acid and glucosinolates. The presence of high levels of erucic acid in traditional rapeseed oil has been associated with detrimental effects in experimental animals. Glucosinolates are located in the seed meal, which is used exclusively as livestock feed. The products of glucosinolate hydrolysis have negative effects on animal production (OECD 2001).

16. The term canola refers to varieties that meet specific standards on the levels of erucic acid and glucosinolates. Canola must contain less than 2% erucic acid in the oil and less than 30 μ moles g⁻¹ of glucosinolates in the meal. Australian canola varieties typically contain levels well below the current standards (OGTR 2011).

4.2 Weediness of non-GM canola

4.2.1 Nature of weediness

17. Weeds are plants that spread and persist outside their natural geographic range or intended growing areas such as farms or gardens and give rise to negative impacts for people or the environment.

18. Negative impacts from weeds may be associated with competitiveness, rambling or climbing growth, toxicity, production of spines, thorns or burrs, or parasitism. The spread and persistence of weeds is a measure of their potential invasiveness, which may give rise to

negative impacts such as reduced establishment of desired organisms, reduced quality of products or services obtained from the land use, reduced access to land, toxicity or increased ill-health of people or other desired organisms and increased degradation of the landscape or ecosystems (National Weed Prioritisation Working Group 2006).

19. The spread and persistence (invasiveness), is determined by complex interactions between a plant and its environment (including availability of water, nutrients and light). A number of measurable properties of plants that may influence spread and persistence include the ability to establish among existing plants, reproductive ability such as time to seeding, amount of seed set and ability for vegetative spread, mode of dispersal, likelihood of long-distance dispersal and tolerance to existing weed management practices (National Weed Prioritisation Working Group 2006).

4.2.2 Weed risk status of canola

20. Baseline information on the characteristics of weeds in general, and the factors limiting the spread and persistence of non-GM canola plants in particular, is given in *The Biology of* Brassica napus *L. (canola)* (OGTR 2011).

21. Canola is considered a major weed in agricultural ecosystems in Australia (Groves et al. 2003). Surveys have shown that canola occurs as a volunteer weed in up to 10% of cereal crops in southern Australia (Lemerle et al. 1996) and similar levels have been reported in Canadian cereal crops (Thomas et al. 1998; Leeson et al. 2005). Canola also occurs as a weed in cropping regions in the USA. (Weed Science Society of America 1992), and it occurs in disturbed habitats along roadsides, railway lines, field margins and waste lands in all countries where it is grown (Norton 2002; Crawley & Brown 2004). However, canola is not considered a significant weed, nor invasive of natural undisturbed habitats in Australia (Dignam 2001; Norton 2002), Canada (Canadian Food Inspection Agency 1994; Warwick et al. 1999; Beckie et al. 2001) or the UK (Crawley et al. 2001).

22. The Australian/New Zealand Standards HB 294:2006 National Post-Border Weed Risk Management Protocol rates the weed risk potential of plants according to properties that strongly correlate with weediness (Virtue et al. 2008). These properties relate to invasiveness, impacts and potential distribution. The weed risk potential of canola has been assessed using methodology based on the National Post-Border Weed Risk Management Protocol (see Appendix 1, OGTR 2011). In summary, canola is considered to:

- have low ability to establish amongst existing plants
- have low tolerance to average weed management practices
- have short time to seeding
- have high annual seed production
- not reproduce by vegetative means
- be unlikely to occasional long distance spread by natural means
- be commonly spread long distance by people
- have limited ability to reduce establishment or yield of desired vegetation
- have low ability to reduce the quality or characteristics of products, diversity or services available from the land use
- have no potential to restrict the physical movement of people, animals, vehicles, machinery and/or water

- have low potential to negatively affect the health of animals and/or people
- have minor or no effect on degradation of the landscape or ecosystems.

23. This is consistent with previous assessments of canola in Australia described above and provides a baseline for the assessment of GM canola.

Section 5 The parental GM canola lines

24. The GM canola proposed for release is the product of conventional breeding between InVigor® canola approved for commercial release under DIR 021/2002, held by Bayer, and Roundup Ready® canola approved for commercial release under DIR 020/2002, held by Monsanto Australia Ltd (Monsanto). These risk assessments are available on the <u>OGTR</u> <u>Website</u> or by contacting the OGTR. Information from these assessments is summarised below, and new information included where available.

25. Seven elite GM canola lines (T45, Topas 19/2, MS1, MS8, RF1, RF2 and RF3) were authorised for commercial release under licence DIR 021/2002. All seven GM canola lines contain a gene conferring tolerance to the herbicide glufosinate ammonium (Table 1 and Table 2). In addition, lines MS1, MS8, RF1, RF2 and RF3 contain genes comprising a hybrid breeding system. Lines Topas 19/2, MS1, RF1 and RF2 also contain an antibiotic resistance gene.

26. The MS and RF lines, and hybrids derived from MS x RF crosses, are covered by the registered trade name InVigor® canola. The hybrid derived from the cross between MS8 and RF3 lines is licensed as InVigor® Hybrid canola for release in Australia under DIR 021/2002. The other lines approved under DIR 021/2002 (T45, Topas 19/2, MS1, RF1 and RF2) were not intended for commercial release in Australia.

27. Roundup Ready® canola has been genetically modified by transformation event GT73 to express two genes conferring tolerance to the herbicide glyphosate (Table 1 and Table 2).

GM canola line	Glufosinate	Glyphosate tolerance	Hybrid breeding	Antibiotic resistance
	ammonium tolerance		system	
GT73	-	Cp4 epsps and goxv247	-	-
T45	pat	-	-	-
Topas 19/2	pat (2 copies)	-	-	nptll (2 copies)
MS1	bar	-	barnase	nptll
MS8	bar	-	barnase	-
RF1	bar	-	barstar	nptll
RF2	bar	-	barstar	nptll
RF3	bar	-	barstar (2 copies)	-

Table 1 The genes introduced into the parental GM canola lines

Table 2	Genetic elements	and	their	origin
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Gene (source)	Protein produced	Protein function	Promoter (source)	Terminator (source)	Additional elements (source)
cp4 epsps (Agrobacterium sp. strain CP4)	CP4 EPSPS	tolerance to the herbicide glyphosate	P-CMoVb (figwort mosaic virus)	E9 3' (Pisum sativum)	AEPSPS/CTP2 (Arabidopsis thaliana)
goxv247 (Ochrobactrum anthropi strain LBAA)	glyphosate oxidoreductase	tolerance to the herbicide glyphosate	PCMoVb (figwort mosaic virus)	E9 3' (Pisum sativum)	SSU1A/CTP1 (Arabidopsis thaliana)

Gene (source)	Protein produced	Protein function	Promoter (source)	Terminator (source)	Additional elements (source)
bar (Streptomyces hygroscopicus)	phosphinothricin acetyl transferase	tolerance to the herbicide glufosinate ammonium	PSsuAra (Arabidopsis thaliana)	3' g7 (Agrobacterium tumefaciens)	-
pat (Streptomyces viridochromogenes)	phosphinothricin acetyl transferase	tolerance to the herbicide glufosinate ammonium	P-35S (Cauliflower mosaic virus)	T-35S (Cauliflower mosaic virus)	-
barnase (Bacillus amyloliquefaciens)	Barnase (RNase)	Male sterility	PTA29 (Nicotiana Tabacum)	3'-nos (Agrobacterium tumefaciens)	-
barstar (Bacillus amyloliquefaciens)	Barstar (RNase inhibitor)	Restoration of fertility	PTA29 (Nicotiana tabacum)	3'-nos (Agrobacterium tumefaciens)	-
nptll (Escherichia coli)	neomycin phosphotransferase	resistance to antibiotics such as kanamycin and neomycin (selectable marker)	P-nos (Agrobacterium tumefaciens)	3'-ocs (Agrobacterium tumefaciens)	-

5.1 The introduced genes, their encoded proteins and their associated effects

5.1.1 Hybrid breeding system genes and their encoded proteins

28. Traditional plant breeding selects for plants with agronomically valuable characteristics. However, repetitive self-pollination of desirable lines can produce progeny that display lowered fitness or vigour when compared to their out-crossing counterparts, a phenomenon termed inbreeding depression. By contrast, when crosses are made between genetically distinct parents, the progeny often outperform the parental lines and are said to display hybrid vigour. Hybrid vigour is commercially advantageous, but ensuring a hybrid cross is technically difficult to achieve, especially when working with species that have both male and female floral organs borne on the same flower and are predominantly self-fertilising, such as canola.

29. To facilitate the production of hybrid canola plants, Bayer has developed a hybrid breeding system that is conferred by expression of the *barnase* and *barstar* genes derived from the common soil bacterium *Bacillus amyloliquefaciens*. *Barnase* encodes a ~12kD ribonuclease (RNase) called BARNASE, and *barstar* encodes a ~10kD RNase inhibitor protein, BARSTAR, which specifically binds to BARNASE and suppresses its activity (Hartley 1988; Hartley 1989).

30. RNases are commonly found in nature and collectively their function is to degrade the messenger ribonucleic acid (mRNA) that allows genetic information to be translated into protein production. This turnover of mRNA is important for regulating the activity of genes. In *B. amyloliquefaciens*, the BARNASE enzyme is secreted extracellularly as a defence mechanism where it degrades the ribonucleic acid of competing organisms. BARSTAR accumulates intracellularly to protect the host cell from the destructive properties of its own ribonuclease enzyme.

31. In the GM canola lines MS1 and MS8, *barnase* is controlled by a promoter that directs gene expression solely within the tapetal cell layer of the anthers. This results in localised

degradation of ribonucleic acid within the tapetal cells prior to microspore development and prevents the production of pollen (Mariani et al. 1990; De Block & De Bouwer 1993). The resulting plants are male-sterile (MS) and can only be fertilised by the pollen of another plant, thereby ensuring the production of hybrid progeny.

32. To reverse the effects of *barnase* expression, GM canola lines have also been generated that contain the *barstar* gene. The introduced *barstar* gene in GM canola lines RF1, RF2 and RF3, is under the control of the same tapetum-specific promoter. Expression of *barstar* has no effect on pollen development and GM canola plants have a normal appearance and viable pollen (Mariani et al. 1992). When a GM line containing *barnase* is crossed with a GM line containing *barstar*, progeny that inherit both genes display completely normal fertility due to the specific inhibition of BARNASE activity by BARSTAR (Mariani et al. 1992). For this reason, GM lines modified with the *barstar* gene expressed from a tapetum-specific promoter are designated as restorers of fertility (RF).

33. Control of fertility by expression of the *barnase* and *barstar* genes is the basis of InVigor® canola hybrids derived from MS x RF crosses, which display hybrid vigour resulting in increased yields over the parental varieties. InVigor® Hybrid canola resulting from the cross between MS8 and RF3 was approved for commercial release under licence DIR 021/2002.

5.1.2 Herbicide tolerance genes and their encoded proteins

Glufosinate ammonium tolerance

34. Glufosinate ammonium is the active ingredient in a number of proprietary broad-spectrum herbicides that have been registered for use in Australia, including Basta®, Finale® and Liberty®. These herbicides function by inhibiting the plant enzyme glutamine synthetase, which is a key enzyme involved in plant nitrogen metabolism. In the absence of glutamine synthetase activity, ammonia accumulates in plant tissues causing inhibition of amino acid biosynthesis, inhibition of photosynthesis and rapid death of the plant (Evstigneeva et al. 2003).

35. The herbicidal component of glufosinate ammonium is the L-isoform of phosphinothricin (PPT). PPT is a component of the antibiotic bialaphos, which is produced naturally by the soil bacteria *Streptomyces hygroscopicus* and *Streptomyces viridochromogenes*. To avoid the toxicity associated with biaphalos production, *S. hygroscopicus* and *S. viridochromogenes* express the biaphalos resistance genes *bar* and *pat*, respectively (Murakami et al. 1986; Thompson et al. 1987; Wohlleben et al. 1988; Strauch et al. 1988). Both the *bar* and *pat* genes encode phosphinothricin acetyl transferase (PAT), an enzyme that acetylates the free amino groups of PPT with high affinity and specificity to render it inactive (Wohlleben et al. 1988; Droge-Laser et al. 1994; OECD 1999b).

The bar and pat genes and their encoded proteins

36. Each of the GM canola lines authorised under licence DIR 021/2002 was modified for tolerance to glufosinate ammonium by the introduction of either the *bar* gene from *S. hygroscopicus* or the *pat* gene from *S. viridochromogenes*. The *bar* and *pat* genes are very similar with an overall identity of 87% at the nucleotide sequence level. Both genes encode PAT proteins of 183 amino acids with 85% amino acid sequence identity, comparable molecular weights (~22 kDa) and similar substrate affinity and biochemical activity (Wehrmann et al. 1996). In fact, the PAT proteins encoded by *bar* and *pat* are so similar as to be functionally equivalent for the purpose of conferring tolerance to glufosinate ammonium (Wehrmann et al. 1996; OECD 1999b).

37. The DNA sequences of both the *pat* and *bar* genes introduced into the GM canola lines approved under DIR 021/2002 were modified for plant-preferred codon usage to ensure optimal expression in canola (European Scientific Committee on Plants 1998a; European Scientific Committee on Plants 1998b; EFSA 2008).

38. The PAT protein produced from the *pat* gene in GM canola lines T45 and Topas 19/2 has exactly the same amino acid sequence as the native protein from *S. viridochromogenes* (European Scientific Committee on Plants 1998a; OECD 1999b).

39. The *bar* gene introduced into the MS and RF GM canola lines was modified by the substitution of the N terminal two codons of the bacterial gene, GTG and AGC, with the codons ATG and GAC, respectively (OECD 1999b; Japanese Biosafety Clearing House 2007). The modification from GTG to ATG does not result in an amino acid change, but serine changes to aspartic acid in the modification from AGC to GAC. However, the function of the PAT gene with this single amino acid substitution remains unchanged (Japanese Biosafety Clearing House 2007).

Glyphosate tolerance

40. Glyphosate is the active ingredient in a number of broad-spectrum systemic herbicides that have been approved for use in Australia and was first marketed as the proprietary herbicide Roundup®. The herbicidal activity of glyphosate is derived from its ability to inhibit the function of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme involved in the shikimate biosynthetic pathway present in all plants, bacteria and fungi.

41. The shikimate pathway enables biosynthesis of aromatic compounds from carbohydrate precursors in a series of seven biosynthetic steps. The penultimate step in the pathway is the condensation of shikimate 3-phosphate and phosphoenol pyruvate to form 5- enolpyruvylshikimate 3-phosphate, a reaction catalysed by EPSPS (reviewed by Herrmann & Weaver 1999). Glyphosate competes with phosphoenol pyruvate for binding to the complex formed between EPSPS and shikimate 3-phosphate. Upon glyphosate binding, the EPSPS:shikimate 3-phosphate complex is very stable and has a slow reversal rate, effectively terminating the shikimate pathway prematurely and preventing biosynthesis of essential aromatic compounds required for plant growth and development, including the amino acids phenylalanine, tyrosine and tryptophan (Dill 2005).

42. Two main approaches have been utilised to generate GM plants that are tolerant to glyphosate-based herbicides: introduction of genes that encode proteins capable of detoxifying the glyphosate molecule; and introduction of genes that encode EPSPS enzymes with reduced affinity for glyphosate (Dill 2005).

43. Roundup Ready® canola line GT73, approved under DIR 020/2002, was modified to contain both a glyphosate detoxifying enzyme, encoded by the *goxv247* gene, and an EPSPS protein with naturally reduced affinity for glyphosate, encoded by the *cp4 epsps* gene.

The goxv247 gene and its encoded protein

44. The *goxv247* gene introduced into Roundup Ready® canola GT73 was isolated from the common soil bacterium *Ochrobactrum anthropi* strain LBAA (formerly *Achromobacter* sp.). It encodes a glyphosate oxidoreductase (GOX) enzyme that inactivates glyphosate by converting it into aminomethylphosphonic acid (AMPA) and glyoxylate (Pipke & Amrhein 1988; Duke 2010). Glyoxylate is a common plant metabolite and AMPA is degraded by several microorganisms (ANZFA 2000).

45. The *goxv247* gene encodes a single polypeptide of 431 amino acids with a molecular mass of 46.1 kD. This gene is a variant of the *O. anthropi gox* gene and has improved affinity for

glyphosate and therefore degrades the herbicide more efficiently. The DNA sequence of *goxv247* was modified for plant-preferred codon usage. The *goxv247* gene varies from the *gox* gene by only 5 nucleotides, and the variant GOXv247 protein is 99% identical to the native GOX enzyme, differing by 3 amino acids.

The cp4 epsps gene and its encoded protein

46. The *cp4 epsps* gene introduced into Roundup Ready® canola GT73 was isolated from the soil bacterium *Agrobacterium* sp. strain CP4 and encodes an EPSPS protein with naturally reduced affinity for glyphosate relative to endogenous plant EPSPS enzymes. The presence of CP4 EPSPS in Roundup Ready® canola allows the plants to complete the shikimate pathway even in the presence of glyphosate.

47. The *cp4 epsps* gene encodes a protein of 47.6 kD consisting of a single polypeptide of 455 amino acids. The nucleotide sequence of the bacterial *cp4 epsps* gene was modified for plant-preferred codon usage, but these nucleotide substitutions did not alter the amino acid sequence of the encoded protein.

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

BARNASE and BARSTAR proteins

48. The *barnase* and *barstar* genes that comprise the hybrid breeding system were derived from the common soil bacterium, *B. amyloliquefaciens*. This bacterium is used commercially as a source of industrial enzyme production, particularly α -amylase, and is also used in the food industry for brewing and bread-making. Although some *Bacillus* species have been implicated as the causal agents of human diseases, *B. amyloliquefaciens* is not known to be allergenic or pathogenic towards humans.

49. GM InVigor® canola lines incorporating the MS and RF hybrid breeding system have been approved for limited and controlled release under DIRs 010/2001, 032/2002, 057/2004, 069/2006 and 104, and for commercial release under DIR 021/2002. Therefore, the toxicity and allergenicity of the BARNASE and BARSTAR proteins have been previously assessed by the Regulator and the assessments concluded that they are unlikely to be toxic or allergenic.

50. No sequence homology was found between BARNASE or BARSTAR and known toxins or allergens (see DIR 069/2006; Van den Bulcke 1997). Further bioinformatic studies using updated databases have confirmed these results (EFSA 2009b). BARNASE and BARSTAR do not have characteristics typical of known protein allergens (Van den Bulcke 1997) and no matches with known IgE epitopes were found (Kleter & Peijnenburg 2002). Both proteins are rapidly degraded in simulated gastric juices (0.32% pepsin and acidic pH) with complete protein degradation within five minutes (Van den Bulcke 1997), showing that these proteins would not survive in the digestive tract.

51. Feeding studies in animals using seed from GM canola lines expressing *barnase* and *barstar* are discussed in Section 5.4.5.

52. Food derived from GM InVigor® canola lines MS1, MS8, RF1, RF2 and RF3, which expresses BARNASE and BARSTAR proteins, has been considered safe for human consumption by FSANZ (ANZFA 2001b), and InVigor® canola has been grown commercially in North America since 1995 without reports of toxicity or allergenicity associated with the introduced genes.

53. BARNASE degrades ribonucleic acid into its component ribonucleotides. Ribonucleotides are ubiquitous in nature and are not considered toxic or allergenic. BARSTAR does not possess enzymatic activity but, instead, exerts its action by binding to the BARNASE enzyme

to form an inactive complex. Therefore, the products of the enzymatic reactions catalysed by the novel proteins are also unlikely to be toxic or allergenic.

PAT protein

54. The *bar* and *pat* genes were obtained from the common soil bacteria *S. hygroscopicus* and *S. viridochromogenes*, respectively. These species of *Streptomyces* are saprophytic, soil-borne microbes that are not considered pathogens of plants, humans or other animals (OECD 1999b).

55. The *bar* and *pat* genes have both been used extensively in the production of GM plants as selectable markers in the laboratory or to provide herbicide tolerance in the field. Consequently, PAT proteins have been used in several GM plants approved by the Regulator for limited and controlled release (for example, see DIR 71/2006, DIR 86/2008 and DIR 100). In addition, GM canola (DIR 021/2002), GM Liberty Link® cotton (DIR 062/2005) and GM WideStrike® cotton (DIR 091) expressing the *bar* or *pat* genes have been approved for commercial release. Therefore, the toxicity and allergenicity of PAT proteins have been previously assessed by the Regulator and the assessments concluded that they are unlikely to be toxic or allergenic.

56. A review of published literature and experimental studies was used to evaluate the safety of the PAT proteins encoded by the *pat* and *bar* genes (Herouet et al. 2005). The authors concluded that there is a reasonable certainty of no harm resulting from including the PAT proteins in human food or animal feed.

57. No sequence homology has been found between PAT and any known toxic or allergenic proteins (Van den Bulcke 1997; Herouet et al. 2005; EFSA 2009b). The PAT proteins do not possess any of the characteristics associated with food allergens and they are not stable in simulated gastric or intestinal fluid conditions (Wehrmann et al. 1996; OECD 1999b; ANZFA 2001b; Herouet et al. 2005) hence the potential for the PAT protein to be a food allergen is minimal (EPA 1997b). In addition, PAT proteins are inactivated by heat, low pH and during processing of canola (Wehrmann et al. 1996; EPA 1997b; European Scientific Committee on Plants 1998a; OECD 1999b).

58. There is no evidence that the PAT proteins encoded by the *bar* and *pat* genes are toxic to either humans or other animals. The potential for PAT to be toxic has been addressed via acute toxicity studies, as detailed in the RARMP for DIR 021/2002. In summary, 14-day acute oral toxicity studies in mice and rats found no treatment-related significant effects (Merriman 1996; Bremmer & Leist 1996). A more recent study found no toxic effect in mice after acute intravenous administration of the PAT proteins at up to 10 mg/kg body weight (Herouet et al. 2005).

59. Feeding studies in animals using seed from GM canola lines containing PAT proteins are discussed in Section 5.4.5.

60. FSANZ has approved the use of food derived from GM plants containing either the *bar* or *pat* gene, including GM canola, cotton, maize, rice and soybean, concluding that the PAT proteins are not toxic (ANZFA 2001a; ANZFA 2001b; FSANZ 2004; FSANZ 2005c; FSANZ 2008).

61. A number of international regulatory bodies have also assessed the PAT proteins expressed in GM plants as safe. These include the United Stated Food and Drug Administration (FDA 1996; FDA 1997; FDA 1998), Health Canada (Health Canada 1997; Health Canada 1999b), the Canadian Food Inspection Agency (Canadian Food Inspection Agency 1995c; Canadian Food Inspection Agency 1996), the European Commission (European Scientific Committee on Plants 1998a) and the European Food Safety Authority (EFSA 2008; EFSA 2009b). The United States Environmental Protection Agency has determined that PAT, and the genetic material necessary for its production, is exempt from the requirement to establish a maximum permissible level for residues in plants (EPA 1997b).

GOXv247 and CP4 EPSPS proteins

62. The *goxv247* gene is derived from *O. anthropi* strain LBAA (formerly *Achromobacter* sp.), a bacterium commonly found in the soil. The *goxv247* gene encodes the GOXv247 protein that differs from the original *O. anthropi* enzyme by three amino acids.

63. *O. anthropi* is an opportunistic human pathogen (Alnor et al. 1994; Mahmood et al. 2000). However, the *gox* gene represents a very small proportion of the pathogen genome and is not, in itself, infectious or pathogenic. The bacterial GOX protein is highly specific for its substrate, glyphosate (OECD 1999a), hence it is unlikely to be involved in human pathogenesis.

64. The *cp4 epsps* gene is derived from another common soil bacteria, *Agrobacterium* sp. strain CP4 (Padgette et al. 1995), which is widespread in the environment and can be found on plant produce (especially raw vegetables). The CP4 EPSPS protein is functionally and structurally similar to EPSPS proteins naturally present in canola and in human food and animal feed derived from other plant and microbial sources (Nair et al. 2002).

65. CP4 EPSPS has been used extensively in GM plants as a selectable marker or a source of field resistance to glyphosate herbicides. Consequently, the Regulator has approved several GM plants expressing *cp4 epsps* for limited and controlled release (for example, see DIR 074/2007, pima cotton; DIR 082, perennial ryegrass and tall fescue; and DIR 101, cotton). The Regulator has also approved GM cotton lines expressing *cp4 epsps* for commercial release under licences DIR 012/2002, DIR 023/2003, DIR 059/2005 and DIR 066/2006. Both the GOXv247 and CP4 EPSPS proteins are present in GM canola approved for limited and controlled release under DIR 011/2001 and DIR 104, and for commercial release under DIR 020/2002. Therefore, the toxicity and allergenicity of GM plants expressing the GOXv247 and CP4 EPSPS proteins has been previously assessed by the Regulator and the assessments concluded that they are unlikely to be toxic or allergenic.

66. The amino acid sequences of both CP4 EPSPS (Mitsky 1993; Harrison et al. 1996) and GOX (Astwood 1995) were compared to the amino acid sequences of known protein toxins and allergens and no significant homology was found. Further bioinformatic studies using updated databases have confirmed that the GOXv247 and CP4 EPSPS proteins do not share any similarity with any known toxins or allergens (EFSA 2009d). The GOXv247 and CP4 EPSPS proteins are readily inactivated by heat and rapidly degraded by simulated mammalian digestive conditions (Harrison et al. 1996; OECD 1999a; Chang et al. 2003).

67. Acute oral toxicity studies using CP4 EPSPS and GOXv247 proteins produced by bacterial expression systems are described in the RARMP for DIR 020/2002. In summary, high doses of the CP4 EPSPS and GOXv247 proteins fed to mice had no adverse effects on food consumption, survival, body weight or gross pathology (Naylor 1994a; Harrison et al. 1996).

68. Feeding studies in animals using seed from GM canola lines containing the CP4 EPSPS and GOXv247 proteins are discussed in Section 5.4.5.

69. Food from Roundup Ready® canola has been approved for human consumption by FSANZ (ANZFA 2000). Food derived from GM soybean, cotton, sugarbeet, maize and

lucerne lines that express the *cp4 epsps* gene have also been considered safe for human consumption by FSANZ (FSANZ 2005a; FSANZ 2005b; FSANZ 2006; FSANZ 2007).

70. A number of international regulatory bodies have also assessed Roundup Ready® canola GT73 with regard to toxicity and allergenicity. These include the United States Environmental Protection Agency (EPA 1996; EPA 1997a), the United Stated Food and Drug Administration (FDA 1995), Health Canada (Health Canada 1999a), the Canadian Food Inspection Agency (Canadian Food Inspection Agency 1995b) and the European Food Safety Authority (EFSA 2009d). These agencies have concluded that the presence of EPSPS and GOX proteins in food does not pose a significant toxicity or allergenicity risk. The EPA considers these proteins as inert ingredients (EPA 1996; EPA 1997a).

5.1.4 Toxicity of herbicide metabolites

71. The potential toxicity of herbicide metabolites is considered by the the Australian Pesticides and Veterinary Medicines Authority (APVMA) in its assessment of a new use pattern for particular herbicides, in this case glyphosate and glufosinate ammonium on InVigor® x Roundup Ready® canola. The issue is summarised below.

Glyphosate metabolites

72. There is no difference in the metabolic fate of glyphosate in non-GM canola and in GM canola expressing *goxv247* and *cp4 epsps*. In the case of CP4 EPSPS, no new metabolic products are formed as the only difference from the native enzyme is the reduced affinity for glyphosate (OECD 1999a).

73. In glyphosate-sensitive plants very little of the glyphosate that is applied would be broken-down. The presence of the GOXv247 protein confers glyphosate tolerance by increasing the rate of breakdown of glyphosate to glyoxylate and aminomethylphosphonic acid (AMPA). Glyoxylate is a common metabolite in plants and forms part of the biochemical pathway that allows synthesis of carbohydrates from fat (the glyoxylate cycle).

74. AMPA is the most frequently detected metabolite of glyphosate in soil, water and plants (Reddy et al. 2008). Despite the faster breakdown of glyphosate, AMPA does not accumulate to higher levels in GM canola expressing GOX than in soybean that does not contain an introduced *gox* gene (Nandula et al. 2007; Duke 2010). AMPA is either non-selectively bound to natural plant constituents, conjugated with naturally occurring organic acids to give trace level secondary metabolites, or further degraded to one-carbon fragments that are incorporated into a variety of natural products and plant constituents (FAO & WHO 1998b).

75. Glyphosate and AMPA have similar toxicological profiles and both exhibit low toxicity (EPA 1997a; Williams et al. 2000; WHO 2005), although AMPA was shown to be genotoxic (able to change DNA) in a recent study using a very sensitive test (Manas et al. 2009). The APVMA sets maximum residue limits (MRLs) for agricultural and veterinary chemicals in agricultural produce, particularly produce entering the food chain. MRLs are set to reflect the legal use of a chemical and to ensure a safe food supply, and are set well below the level that would be harmful. The residue definition for glyphosate includes the metabolite AMPA (APVMA 2011).

Glufosinate ammonium metabolites

76. The herbicide glufosinate ammonium is comprised of a racemic (equal) mixture of the Land D- enantiomers. The L- enantiomer is the active constituent and acts by inhibiting the enzyme glutamine synthetase. D-glufosinate ammonium does not exhibit herbicidal activity and is not metabolised by plants (Ruhland et al. 2002). 77. The PAT enzyme, encoded by either the *bar* or *pat* gene, inactivates the L-isomer of glufosinate ammonium by acetylating it to N-acetyl- L- glufosinate ammonium (NAG), which does not inhibit glutamine synthetase (Droge-Laser et al. 1994; OECD 2002). This metabolite is not found in non-GM plants.

78. The metabolism of glufosinate ammonium in tolerant GM plants and in non-GM (non-tolerant) plants has been reviewed (FAO & WHO 1998a; OECD 2002). In non-GM plants the metabolism of glufosinate ammonium is low to non-existent because of plant death due to the herbicidal activity. However, some metabolism does occur (Muller et al. 2001) and is different to that in GM plants expressing the PAT protein (Droge et al. 1992).

79. Two pathways for the metabolism of glufosinate ammonium in non-GM plants have been identified. The first step, common to both pathways, is the rapid deamination of L-phosphinothricin to the unstable intermediate 4-methylphosphonico-2-oxo-butanoic acid (PPO). PPO is then metabolised to either:

- 3-methyl-phosphinico-propionic acid (MPP, sometimes referred to as 3-hydroxy-methyl phosphinoyl-propionic acid) which may be further converted to 2-methyl-phosphinico-acetic acid (MPA); or
- 4-methylphosphonico-2-hydroxy-butanoic acid (MHB), which may be further converted to 4-methylphosphonico-butanoic acid (MPB), a final and stable product (Droge-Laser et al. 1994; Ruhland et al. 2002; Ruhland et al. 2004).

80. The main metabolite in non-GM plants is MPP (Muller et al. 2001; OECD 2002).

81. The metabolism of glufosinate ammonium has been investigated in GM herbicide-tolerant canola, maize, tomato, soybean and sugar beet (FAO & WHO 1998a; OECD 2002). The major residue present in the GM crops after glufosinate ammonium herbicide application was NAG, with lower concentrations of glufosinate ammonium and MPP. Studies using cell cultures of GM canola gave similar results, with NAG being the major metabolite (Ruhland et al. 2002).

82. Both NAG and MPP are less toxic than glufosinate ammonium, which itself has low toxicity (OECD 1999b; OECD 2002; EFSA 2005).

5.1.5 The antibiotic resistance marker gene (nptll) and its encoded protein

83. The GM canola lines Topas 19/2, MS1, RF1 and RF2 authorised under DIR 021/2002 contain the antibiotic resistance marker gene neomycin phosphotransferase type II (*nptII*).

84. The *nptII* gene, encoding the enzyme neomycin phosphotransferase, was derived from the common gut bacterium *Escherichia coli* and confers resistance to antibiotics such as kanamycin and neomycin on GM plant cells. The *nptII* gene was used during initial development of the GM plants in the laboratory to select plant cells containing the introduced genes.

85. The *nptII* gene is used extensively as a selectable marker in the production of GM plants (Miki & McHugh 2004). As discussed in previous DIR RARMPs, and in more detail in the RARMPs for DIR 070/2006 and DIR 074/2007, regulatory agencies in Australia and in other countries have assessed the use of the *nptII* gene in GM plants as not posing a risk to human or animal health or to the environment. A recent detailed evaluation of *nptII* in terms of human safety by the European Food Safety Authority concluded that the use of the *nptII* gene as a selectable marker in GM plants (and derived food or feed) does not pose a risk to human or animal health or to the environment (EFSA 2009a).

5.2 The regulatory sequences

86. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. Information on the promoters, terminators and other regulatory genetic elements used to control expression of the introduced genes in the parental GM canola lines are listed in Table 2 (above) and described below.

5.2.1 Regulatory sequences for the expression of the introduced bar gene

87. Expression of *bar* is controlled by the plant promoter *PSsuAra* from the *Arabidopsis thaliana ats1A* gene, which encodes a ribulose-1,5-bisphosphate carboxylase small subunit (rbcS) peptide (Krebbers et al. 1988). The *PSsuAra* promoter directs gene expression predominantly in green plant tissues (Krebbers et al. 1988; De Almeida et al. 1989).

88. The mRNA terminator for the *bar* gene is derived from the 3' non-translated region of the T-DNA gene 7 (3'g7) of *Agrobacterium tumefaciens* (Dhaese et al. 1983).

5.2.2 Regulatory sequences for the expression of the introduced pat gene

89. The *pat* gene is controlled by the constitutive 35S promoter and 35S terminator from Cauliflower mosaic virus (CaMV) (Odell et al. 1985) in both lines T45 and Topas 19/2. The CaMV 35S promoter has been used extensively in plant transformation studies (Sunilkumar et al. 2002; Squires et al. 2007). The 35S terminator has also been widely used in GM plants (Mitsuhara et al. 1996).

5.2.3 Regulatory sequences for the expression of the introduced barnase and barstar genes

90. The *barnase* and *barstar* genes are controlled by PTA29, a 1.5 kb promoter fragment derived from the tobacco (*Nicotiana tabacum*) *TA29* gene (Goldberg 1988; Seurinck et al. 1990). *TA29* is expressed specifically in the tapetal cells of tobacco anthers (Koltunow et al. 1990) and anther-specific expression was reproduced when the PTA29 promoter was used to drive transgene expression in tobacco and canola (Mariani et al. 1990; De Block & De Bouwer 1993).

91. As discussed in Section 5.1.1, expression of the *barnase* and *barstar* genes in GM InVigor canola lines occurs only in the tapetum cell layer of the pollen sac during anther development, resulting in production of cytotoxic RNase, and inactivation of the same RNase activity, respectively (Mariani et al. 1990; Mariani et al. 1992; De Block & De Bouwer 1993).

92. For both genes, the terminators are derived from the 3' non-translated region of the nopaline synthase gene (3' *nos*) from *A. tumefaciens* (Depicker et al. 1982). The *nos* terminator has been used in a wide variety of constructs for plant genetic modification (Reiting et al. 2007).

5.2.4 Regulatory sequences for the expression of the introduced cp4 epsps and goxv247 genes

93. Expression of *cp4 epsps* and *goxv247* is driven by the Figwort mosaic virus (FMV) promoter P-CMoVb (Richins et al. 1987; Gowda et al. 1989; Sanger et al. 1990). P-CMoVb is a constitutive promoter which directs gene expression in all plant parts (Sanger et al. 1990; Maiti et al. 1997). The P-CMoVb promoter is thought to be equivalent to the 35S promoter from CaMV, despite low sequence conservation overall between these two promoters. This conclusion was reached because the two promoters occupy similar positions in their respective viral genomes, both increase in strength with increasing sequence length, and the core promoters have significant sequence homology (Sanger et al. 1990).

94. For both genes, the terminators are derived from the 3' untranslated region of the *E9* gene (*E9* 3') from *Pisum sativum*, which encodes a ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit (rbcS) peptide (Coruzzi et al. 1984; Morelli et al. 1985).

95. The *cp4 epsps* and *goxv247* genes are each fused to a chloroplast transit peptide sequence to target the proteins to the chloroplasts (the site of aromatic amino acid biosynthesis). Transit peptides occur naturally in plants and function to direct proteins into specific organelles. In plants, EPSPS is synthesised as a pre-protein containing a transit peptide by free cytoplasmic ribosomes. The pre-protein is transported into the chloroplast stroma where the transit peptide is cleaved and rapidly degraded leaving the mature enzyme (Bartlett et al. 1982; della-Cioppa et al. 1986).

96. The *cp4 epsps* gene is fused to a chloroplast transit peptide from the *A. thaliana epsps* gene (AEPSPS/CTP2) (Klee et al. 1987). The *goxv247* gene is fused to a chloroplast transit peptide from an *A. thaliana* gene encoding an *rbcS* peptide (SSU1A/CTP1) (Krebbers et al. 1988).

5.2.5 Regulatory sequences for the expression of the introduced nptll gene

97. Expression of the *nptII* gene in GM canola lines Topas 19/2, MS1, RF1 and RF2 is controlled by the nopaline synthase promoter (P-*nos*) from *A. tumefaciens* (Bevan et al. 1983). The terminator is derived from the 3' non-translated region of the octopine synthase gene (3' *ocs*) from *A. tumefaciens* (Dhaese et al. 1983).

5.3 Method of genetic modification

98. The GM canola proposed for release is the product of conventional breeding between InVigor® canola lines approved for commercial release under DIR 021/2002 and Roundup Ready® canola line GT73 approved for commercial release under DIR 020/2002.

99. All of the parental GM canola lines were generated by *Agrobacterium*-mediated transformation using the plasmids described in Table 3 (della-Cioppa et al. 1987; De Block et al. 1989; FAO & WHO 1998a). *A. tumefaciens* is a soil bacterium that causes gall formation on a wide range of plant species. The gall is induced by transfer of hormone-producing genes from the bacterial cell into the plant genome. The genes are carried on an extrachromosomal, circular DNA molecule found within the bacterial cell called a Tumour-inducing (Ti) plasmid. During the infection process, only a section of the Ti plasmid known as the Transfer DNA (T-DNA) is transferred to the plant.

100. Molecular biologists have studied the infection and T-DNA transfer process of *A. tumefaciens* for many years and have used this natural process to facilitate genetic modification of plants. *A. tumefaciens* Ti plasmids have been produced that lack the genes responsible for tumour formation (disarmed plasmids) and instead enable genes of interest to be inserted between the T-DNA border sequences. When used to infect plants, *A. tumefaciens* cells carrying such plasmids cannot produce a tumour but will transfer the T-DNA sequence carrying the genes of interest into the plant cell where they stably integrate into the plant genome (Bevan 1984; Klee & Rogers 1989).

Plasmid name	GM canola line	Introduced Genetic Elements
pHOE4/Ac(II)	T45	- 35S promoter from CaMV
		- pat gene from S. viridochromogenes
		- 35S terminator from CaMV
pOCA18/Ac	Topas 19/2	- P-nos promoter from A. tumefaciens
		- nptll gene from E. coli
		- 3'-ocs terminator from A. tumefaciens
		 colE1 origin of replication from E. coli*
		 35S promoter from CaMV
		 pat gene from S. viridochromogenes
		 35S terminator from CaMV
		 cos site from bacteriophage lambda*
pTTM8RE	MS1	 3'-ocs terminator from A. tumefaciens
		- nptll gene from E. coli
		 P-nos promoter from A. tumefaciens
		- PTA29 promoter from <i>N. tabacum</i>
		- barnase gene from B. amyloliquetaciens
		- 3'-nos from A. tumetaciens
		- PSsuAra promoter from A. thaliana
		- bar gene from S. hygroscopicus
	MOO	- 3 g/ terminator from A. tumetaciens
	IVIS8	- PTA29 promoter from N. tabacum
		- barnase gene from B. arrivioliquelaciens
		- 5-nos nom A. tumeraciens
		- Fosuara promoter mont A. manana
		-3° a7 from A tumefaciens
nTVF74RF	RE1 and RE2	- 3'-ocs terminator from A tumefaciens
		- nntll gene from E coli
		- P-nos promoter from A tumefaciens
		- PTA29 promoter from <i>N. tabacum</i>
		- barstar gene from <i>B. amvloliguefaciens</i>
		- 3'-nos from A. tumefaciens
		- PSsuAra promoter from A. thaliana
		- bar gene from S. hygroscopicus
		- 3' g7 terminator from A. tumefaciens
pTHW118	RF3	- PTA29 promoter from <i>N. tabacum</i>
		- barstar gene from B. amyloliquefaciens
		- 3'-nos gene from A. tumefaciens
		 PSsuAra promoter from A. thaliana
		 bar gene from S. hygroscopicus
		- 3' g7 from A. tumefaciens
PV-BNGT04	Roundup Ready®	- P-CMoVb promoter from FMV
	canola GT73	- SSU1A/CTP1 sequence from A. thaliana
		- goxv247 gene from <i>O. anthropi</i>
		- 3° E9 from P. sativum
		- P-CMOVb promoter from FMV
		- CTP2 sequence of the epsps gene from A. thallana
		- cp4 epsps gene from Agrobacterium strain CP4
		I = 3 E9 IIOIII P Salivului

Table 3: List of plasmids used to generate the parental GM canola lines

* The colE1 and cos sequences are of non-eukaryotic origin and will not function in the plant.

5.4 Toxicity/allergenicity of the parental GM canola lines

101. The toxicity of the parental GM canola lines to people and to other organisms, including insects, birds, mice, rabbits, kangaroos and grazing livestock, was considered in the RARMPs for DIRs 020/2002 and 021/2002. The safety of feed produced from the parental GM canola lines for livestock was also considered. The Regulator concluded that the parental

GM canola lines are as safe as non-GM canola. These assessments, plus new or updated information, are summarised below.

5.4.1 Toxicity/allergenicity to humans

102. Canola oil is the only fraction used in human food. Due to the extensive processing applied during canola oil extraction and refinement, no protein, including any novel proteins, would be expected to be detected in canola oil (ANZFA 2001b). Therefore, oil derived from the GM canola proposed for release would not contain any of the novel proteins.

103. Food derived from all of the parent lines used to generate the GM canola proposed for release has been approved for human consumption in Australia (ANZFA 2000; ANZFA 2001b) and other countries (see Section 5.1.3). These approvals also cover the GM InVigor® x Roundup Ready® canola proposed for release.

104. People could be exposed to pollen containing the introduced genes, either through occupational exposure or in honey. Canola is commonly utilised as a source of nectar and pollen for commercial honey production by honeybees. However, only low amounts of canola pollen are present in honey. The percentage dry weight of canola pollen per wet weight of honey that is produced from hives placed in canola fields is only 0.2 % (Hornitzky & Ghalayini 2006). If the honey is sieved or filtered the pollen content is further reduced (discussed in Malone 2002).

105. The introduced proteins are expressed only at low levels in plant tissues. No expression of the *bar*, *barnase*, *barstar* or *nptII* genes has been detected in pollen from the InVigor® canola parental lines (see Chapter 1, Section 6.2.2). Therefore, the level of exposure of people to the introduced proteins in pollen would be extremely low. Most importantly, none of the introduced proteins are toxic or allergenic, and the introduced genes were all isolated from common bacteria, that are widespread and prevalent in the environment (see Section 5.1.3).

5.4.2 Toxicity to animals, including livestock

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

107. The production of canola meal involves a number of processes, including seed flaking, heating, mechanical crushing to remove oil, solvent extraction of oil, desolventising and toasting of the meal. Toasted canola meal is the most common fraction used as animal feed, although some meal (20%) is physically extracted without added heat. A small amount (5%) of canola meal available in Australia is from cold-pressed seed (Mailer 2004).

108. As discussed in Section 4, glucosinolates and erucic acid are naturally occurring toxicants in canola seed. Glucosinolates remain in the canola meal after oil extraction while erucic acid is removed with the oil fraction during processing of the seed. Industry standards require canola meal to contain less than 30 μ moles g⁻¹ of glucosinolates. Compositional analyses demonstrate that the levels of erucic acid and glucosinolates in Roundup Ready® and InVigor® canola lines are below standard levels and do not vary significantly from their parental cultivars or other commercially available canola.

109. The introduced genes were all isolated from common soil bacteria that are widespread and prevalent in the environment. The *barnase* and *barstar* genes are not expressed in the seeds or leaves of InVigor® canola, therefore livestock would not be exposed to these

proteins. The *nptII*, *pat*, *cp4 epsps* and *goxv247* genes are only expressed at low levels in GM canola seed and/or leaves. The amount of each protein is further reduced during processing of the seed that results in the production of meal (ANZFA 2000; ANZFA 2001b).

110. The PAT, CP4 EPSPS and GOXv247 proteins are not toxic, even at high doses, as demonstrated by acute oral toxicity studies in animals (see Section 5.1.3). While the assessment of the toxicity of the herbicide metabolites to non-target organisms is the responsibility of the APVMA, the major metabolites of glufosinate ammonium and glyphosate are also not toxic (see Section 5.1.4). The composition of the parental GM canola lines does not differ significantly from non-GM canola (see Section 6.2.4) other than by the presence of the introduced proteins, and feeding studies on a range of organisms demonstrate that there are no anti-nutritional effects of the genetic modifications in the parental GM canola lines (see Section 5.4.5).

111. The parental GM canola lines have been assessed and approved for use in animal feed by regulatory agencies in Europe, Canada and the USA (FDA 1995; Canadian Food Inspection Agency 1995b; Canadian Food Inspection Agency 1996; FDA 1996; FDA 1997; FDA 1998; European Scientific Committee on Plants 1998b; EFSA 2004). Roundup Ready® canola, glufosinate ammonium tolerant canola and/or InVigor® hybrid lines have been approved for use in animal feed since 1995 and there have been no reports of adverse effects to livestock fed these GM canola lines.

5.4.3 Toxicity to honey bees

112. As honey bees are a major pollinator of canola, the potential effects of the genetic modifications in the parental GM canola lines on honey bees were considered in detail in the RARMPs for DIR 020/2002 and 021/2002. Studies cited in these documents did not find any negative impacts on bees foraging on Roundup Ready® canola, InVigor® canola, or other GM glufosinate ammonium tolerant canola plants (USDA-APHIS 1999a; Canadian Food Inspection Agency 1995a; Canadian Food Inspection Agency 1995c; USDA-APHIS 1998; European Scientific Committee on Plants 1998a; European Scientific Committee on Plants 1998a; European Scientific Committee on Plants 1998b; Malone & Pham-Delegue 2001; Malone 2002; Pham-Delegue et al. 2002).

113. Two more recent studies have shown reduced abundance of bees in GM herbicide tolerant canola compared to non-GM canola (Haughton et al. 2003; Morandin & Winston 2005). In both studies, the authors propose that the differences were an indirect result of herbicide treatments that effectively reduced weed numbers and diversity in the GM fields, consequently reducing forage for bees.

114. A number of regulatory agencies have assessed whether the parental GM canola lines have any increased toxicity to non-target organisms as a result of the genetic modifications. In its assessments of Roundup Ready® canola and GM canola lines MS8 and RF3, the USDA-APHIS determined that the GM canola lines would not harm threatened or endangered species or other organisms, such as bees, that are beneficial to agriculture (USDA-APHIS 1999a; USDA-APHIS 1999b; USDA-APHIS 1999c). The Canadian Food Inspection Agency (CFIA) concluded that the unconfined release of Roundup Ready® canola and GM canola lines MS8 and RF3 would not result in altered impacts on interacting organisms, and that their potential impact on biodiversity is equivalent to that of currently commercialised canola varieties (Canadian Food Inspection Agency 1995b; Canadian Food Inspection Agency 1996).

5.4.4 Toxicity to soil microbes

115. Several studies have investigated the effects of growing GM glyphosate tolerant canola or GM glufosinate ammonium tolerant canola on soil microbes. These studies were

described in detail in the RARMPs prepared for DIR 020/2002 and 021/2002. Slightly altered microbial communities in the rhizosphere of GM canola plants have been reported. These differences were minor and generally not sustained after removal of the GM plants (Dunfield & Germida 2001; Gyamfi et al. 2002; Dunfield & Germida 2003).

116. Recent studies have confirmed the lack of permanent effects on soil biota by GM glyphosate tolerant crops. For example, no permanent effects on soil biota were observed in a series of experiments designed to estimate the effect of glyphosate tolerant soybean and maize, and their management, on the abundance of detritivorous soil biota and crop litter decomposition (Powell et al. 2009). While significant effects were observed in a few of the measured groups, in most cases the effects were only observed in the first year of the study and were not consistent across sample dates or across the four study years. The most frequent effect of the glyphosate tolerant herbicide system was a transient shift toward more fungal biomass relative to bacterial. The genetic modification in the soybean and maize had little effect on litter decomposition, however the use of glyphosate did reduce decomposition of surface (but not buried) litter.

117. In a field experiment conducted at six sites in Canada, repeated plantings of glyphosate tolerant wheat and glyphosate tolerant canola grown in rotation had only minor and inconsistent effects on soil microorganisms over a wide range of growing conditions and crop management regimes (Lupwayi et al. 2007). As is the case for many studies that show an effect of herbicide resistant cropping systems on microbial communities, the effects of the glyphosate tolerance trait and the herbicide applications were not separated in this study. Application of herbicides can affect proportions of soil microbes (for example, see Becker et al. 2001; Gyamfi et al. 2002; Kremer & Means 2009; Mijangos et al. 2009).

118. Crop type (GM or non-GM) made no difference to the abundance or structure of microbial communities in a study designed to separate the effects of GM glyphosate tolerant maize from the use of glyphosate on denitrifying bacteria and fungi (Hart et al. 2009). The GM maize in this study expressed the cp4 epsps gene, and the authors note that the use of a protein derived from a common soil bacterium may affect soil microbial communities less than modifications that introduce novel proteins into the soil. The genes for herbicide tolerance and a hybrid breeding system in this DIR 108 application were all isolated from common soil bacteria.

5.4.5 Feeding Studies

119. Several feeding studies have been undertaken with the parent lines used to generate the GM canola proposed for release. Data from these studies were submitted in conjunction with the applications for licences DIR 020/2002 and 021/2002, and fully assessed in the RARMPs for these licences. A brief summary of these studies, along with new or updated information, is provided below.

InVigor® canola

120. Two feeding studies were conducted in rabbits to investigate the nutritive value of canola seed of hybrids derived from crosses of MS1 x RF1 (ANZFA 2001b) and MS8 x RF3 (Maertens et al. 1996). No significant differences in feed intake, feed efficiency, weight gain or final weight of the rabbits were observed between the GM canola diet and the non-GM canola diet, indicating that the nutritional value of the GM hybrid canola was comparable to the non-GM parental line (ANZFA 2001b).

121. Similarly, in a study of canaries fed seed from either MS1 x RF1 hybrids or non-GM canola, no differences in food consumption, behaviour or body weight were observed between the GM and non-GM diets (Canadian Food Inspection Agency 1995c).

122. One feeding study involving broiler chickens fed seed from GM canola line Topas 19/2 was described in the RARMP for DIR 021/2002. There were no differences between the chickens fed Topas 19/2 canola seed and those fed non-GM canola seed for any of the measured parameters, including body weight, body weight gain, feed intake, mortality rate and carcass characteristics at post-mortem (Leeson 1999).

123. Subsequently, another 42-day feeding study in broiler chickens has been reported (EFSA 2009b). This study was carried out on 420 male broiler chickens, which were divided into three groups and fed diets containing 10% GM canola hybrid MS8 x RF3 that had been either treated with glufosinate ammonium or untreated, or a diet containing 10% non-GM canola. No significant differences were observed in any of the parameters measured (animal health, survival, feed intake, weight gain, feed conversion and carcass and muscle weight), showing that MS8 x RF3 GM hybrid canola is nutritionally equivalent to non-GM canola (EFSA 2009b).

Roundup Ready® canola

124. Broiler chickens were used to compare diets containing Roundup Ready® canola GT73, the parental non-GM canola line, and six commercially available canola lines (Taylor et al. 2004; Stanisiewski et al. 2002). Values obtained for a range of parameters were similar across the diets demonstrating that Roundup Ready® canola GT73 is as nutritious as non-GM canola.

125. Similarly, feeding studies in bobwhite quail chicks (Campbell et al. 1993; Campbell & Beavers 1994), trout (Brown et al. 2003), lambs (Stanford et al. 2002; Stanford et al. 2003) and pigs (Aalhus et al. 2003; Caine et al. 2007) found no significant differences between animals fed Roundup Ready® canola GT73 containing diets and control diets, supporting the conclusion that Roundup Ready® canola meal is nutritionally equivalent to non-GM canola meal (EFSA 2009d).

126. Three one-month feeding studies were conducted on rats (Naylor 1994b; Nickson & Hammond 2002). No changes attributable to the genetic modification were observed. FSANZ thoroughly considered these studies in its assessment of Roundup Ready[®] canola GT73 before reaching the conclusion that 'oil derived from glyphosate-tolerant canola GT73 is as safe for human consumption as oil from other commercial canola varieties' (ANZFA 2000).

5.5 Weediness of the parental GM canola lines

127. The risk of the genetic modifications in the parental GM canola lines making them more invasive or persistent than non-GM canola in Australia was assessed in the RARMPs for licences DIR 020/2002 and 021/2002. The Regulator concluded that the parental GM canola lines are no more invasive or persistent than non-GM canola. A brief summary of this assessment, along with new or updated information, is provided below.

5.5.1 Spread and persistence in the environment

128. Although conventional canola has a number of weedy characteristics, it is a poor competitor and is not invasive. Canola is not a significant weed in habitats outside agricultural areas and does not pose a serious threat to the environment and biodiversity. The risk that the Roundup Ready® or InVigor® canola will be more likely to spread and persist in the environment and cause more harm to the environment than non-GM canola is negligible.

129. There is no evidence to show that the introduced genes increase the potential weediness of the plants. The germination, seed dormancy and fitness traits such as sensitivity to other herbicides, disease resistance, stress adaptation and competitiveness for Roundup

Ready® or InVigor® canola fall within the range of non-GM open-pollinated and hybrid canola varieties.

130. The hybrid vigour displayed in InVigor® canola hybrids is not a function of the genetic modification that can be transferred as a single trait, but is a result of breeding two genetically distinct parents. In general, hybrid vigour manifested in the F1 generation declines in subsequent generations (Falconer & Mackay 1996).

131. InVigor® canola hybrids have displayed yield increases of 10-20% over non-GM open pollinated varieties in Australia and greater than 20% in Canada (Clayton et al. 1999; Zand & Beckie 2002; Bayer CropScience 2003; Harker et al. 2003). However, the superior seedling emergence and increased seed numbers (Clayton et al. 1999; Bayer CropScience 2003; Harker et al. 2003) does not lead to the expected increase in volunteers in commercial fields in Canada (Beckie & Owen 2007) or in trials in the UK, due to greater uniformity in ripening (Crawley et al. 1993; Sweet 1999; MacDonald & Kuntz 2000). Volunteers of herbicide resistant hybrids are no more invasive of agricultural or disturbed habitats than volunteers of herbicide resistant open pollinated canola (Beckie & Owen 2007; Warwick et al. 2009). Data obtained in Australia indicate that the vigour exhibited by InVigor® canola hybrids falls within the range of vigour exhibited by non-GM hybrid and open pollinated varieties of canola grown commercially (see DIR 021/2002).

132. GM herbicide tolerant canola has no altered weedy or invasiveness potential (Hall et al. 2005; Warwick et al. 2009). The genetic modifications do not provide Roundup Ready® or InVigor® canola with an ecological advantage over conventional canola except in the presence of glyphosate or glufosinate ammonium, respectively. Glyphosate is widely used for weed control in broad-acre agriculture, horticulture and other weed management situations. Glufosinate ammonium is not registered for use in any broad-acre crop except on Bayer's GM InVigor® canola and GM Liberty Link® cotton varieties. It is used in viticulture and horticulture but is rarely used in non-agricultural areas.

133. Roundup Ready® and InVigor® canola are only tolerant to glyphosate or glufosinate ammonium, respectively, and their susceptibility to other herbicides is no different to non-GM canola. GM canola volunteers can be managed and controlled using alternative herbicides assessed and approved by the APVMA as well as other non-chemical management practices in the same manner as non-GM canola volunteers. The impact of such changes is considered to be primarily an agricultural production issue with a potential economic impact.

Agricultural environments

134. The risk that Roundup Ready® or InVigor® canola will be more invasive or persistent in the agricultural environment than non-GM canola and result in a more detrimental environmental impact was assessed as negligible.

135. Non-GM canola is primarily dispersed by human activities (harvest, transport) (Agrisearch 2001; Crawley & Brown 2004; von der Lippe & Kowarik 2007) and this would be the case with Roundup Ready® or InVigor® canola.

136. Canola seed can be dispersed by grazing animals (eg sheep, Stanton et al. 2003) or wild birds (Twigg et al. 2008; Woodgate et al. 2011). Wind may move plant material from windrows. This material is generally caught in the next windrow or trapped by the remaining stubble, but can on occasions be moved over greater distances and cross boundary fences. There is no evidence that dispersal of seed would be different for GM canola.

137. Volunteer canola (non-GM and GM) represents a weed of agricultural production systems (Legere et al. 2001; Beckie et al. 2001; Martens 2001; Simard & Legere 2001;

Simard et al. 2002). There are no differences between Roundup Ready® or InVigor® canola and non-GM canola with respect to the intrinsic characteristics contributing to spread and persistence, such as seed production, shattering or dormancy, and competitiveness. Roundup Ready® and InVigor® canola varieties have been grown commercially in Canada since the mid-1990's and there is no indication that they are more intrinsically persistent than non-GM canola (Derksen et al. 1999; Norris et al. 1999; MacDonald & Kuntz 2000; Crawley et al. 2001).

138. Non-GM canola can display secondary dormancy and can persist for several years as an agricultural weed, particularly as volunteers following canola crops resulting from harvest losses (Lutman 1993; Pekrun et al. 1998; Gruber et al. 2005; Harker et al. 2006; Gruber et al. 2008; Gruber et al. 2010). This appears to apply equally to glyphosate or glufosinate ammonium tolerant canola (Fredshavn & Poulsen 1996; Norris et al. 1999; Simard et al. 2002; Salisbury 2002c; Beckie & Owen 2007). Gulden et al (2000) found no significant differences between dormancy of Roundup Ready® canola or other herbicide tolerant canola, including InVigor® cultivars, and non-GM canola, but did find significant differences between varieties, indicating that the parental genotype is an important factor in the degree of dormancy (Gulden et al. 2000).

139. Roundup Ready® and InVigor® canola only have a survival advantage in the presence of glyphosate or glufosinate ammonium, respectively. Studies of glufosinate ammonium tolerant canola lines and non-GM cultivars grown in monoculture or in a mixture with barley showed no differences in competitive ability (Poulsen et al. 1999). Another study showed that glufosinate ammonium tolerant oilseed-rape showed significantly lower seedling establishment when compared with non-GM canola lines in six out of twelve cases and significantly higher in two cases (Crawley et al. 2001).

140. Glyphosate is commonly used in broad-acre cropping for pre-emergent weed control prior to planting. Glyphosate would not be effective in controlling canola volunteers in situations where Roundup Ready® canola had been grown previously. The presence of Roundup Ready® canola volunteers in agricultural or disturbed habitats has implications for the choice of herbicide(s) in situations where glyphosate is the principal weed control strategy.

141. Roundup Ready® and InVigor® canola are as susceptible to all other herbicides except glyphosate or glufosinate ammonium, respectively, as non-GM canola. The GM canola volunteers can be controlled by using the variety of other herbicides assessed and approved by the APVMA as well as non-chemical management methods currently used to control non-GM canola.

Non-cropped disturbed habitats

142. Canola is found in low densities in non-cropped disturbed situations, such as grassy road verges (MacDonald & Kuntz 2000; Norton 2003). The available evidence supports the conclusion that the GM canola lines approved for release under DIRs 020/2002 and 021/2002 pose no greater weed threat than non-GM canola in non-cropped disturbed habitats.

143. Due to its primary colonising nature, canola can take advantage of disturbed land (Salisbury 2002c). Canola plants are often observed growing near transport routes and at field margins (Agrisearch 2001; Crawley & Brown 2004; von der Lippe & Kowarik 2007; Nishizawa et al. 2009). In Australia and Canada, roadside canola populations are thought to be reliant on re-supply of seed from seed spillage during harvest and transport operations rather than forming self-sustaining weed populations (Salisbury 2002c; Gulden et al. 2008). However, canola is a poor competitor and will be displaced unless the habitats are disturbed
on a regular basis (OECD 1997; Beckie et al. 2001; Salisbury 2002c). Herbicide tolerant crops in general are not considered noxious weeds and have not been more invasive in disturbed areas (Beckie et al. 2006; Beckie & Owen 2007; Warwick et al. 2009).

144. The Conservation Council of Western Australia recently published a survey of roadside canola plants conducted by the Conservation Council (WA) Citizen Science Program, Esperance Local Environmental Action Forum (LEAF) and GM Cropwatch⁷. The survey was conducted in September 2011 to determine the frequency and distribution of GM Roundup Ready® canola plants in the Esperance region of WA after one year of commercial production. Two GM positive plants were detected among 190 canola plants collected and tested, representing 1.05%⁷. The area sown to GM canola was around 8% of the total canola crop in WA in 2010 (DAFWA 2010).

145. Roundup Ready® and InVigor® canola volunteers occurring in disturbed environments will not have any competitive advantage over conventional canola in the absence of glyphosate or glufosinate ammonium selection (Wilkinson et al. 1995; Senior & Dale 2002; Warwick et al. 2009).

146. Glufosinate ammonium is registered for use in commercial and industrial areas, rights-of-way and other non-agricultural areas under the trade names Basta® and Finale®, but it is not widely used for weed control by local councils and Road and Rail authorities (Dignam 2001).

147. Glyphosate is widely used in weed control operations in disturbed environments such as roadsides. However, while glyphosate is very effective in controlling grasses, it does not always achieve complete control of established broadleaf weeds. A mixture of herbicides (commonly referred to as 'spiking') may be used to ensure complete control of broadleaf weeds. Management of Roundup Ready® canola in roadsides and other disturbed habitats can be achieved by the variety of management strategies available, including a range of alternative herbicides to glyphosate, tank mixing of other herbicides with glyphosate, and non-chemical management methods such as mowing, cultivation, burning and grazing.

Undisturbed environments

148. Canola is considered a weed of agricultural and disturbed habitats, but is of minor significance to natural ecosystems (Groves et al. 2003). The genotypes used for commercial canola cultivation are bred for maximum production in managed environments in which optimal water and nutrient availability is ensured. In natural environments where water and nutrient availability are limited, canola is considered a poor competitor compared with native species (Hall et al. 2005; Oram et al. 2005; Canadian Food Inspection Agency 2007). When roadsides were surveyed for the presence of GM canola, it was only found in the 5 m closest to the edge of the road, but not further away from the road (Crawley & Brown 2004).

149. The available evidence supports the conclusion that the GM canola lines approved for release under DIRs 020/2002 and 021/2002 pose no greater weed threat resulting in adverse impacts on the environment than non-GM canola in undisturbed natural habitats. GM herbicide tolerant crops in general are not considered noxious weeds and have not been more invasive in natural ecosystems (Beckie et al. 2006; Beckie & Owen 2007; Warwick et al. 2009).

150. Roundup Ready® and InVigor® canola do not have any competitive advantage in the absence of glyphosate or glufosinate ammonium, respectively. Even if glufosinate ammonium

⁷ <u>Source</u>, accessed 9 November 2011

and/or glyphosate tolerant canola did establish in undisturbed natural habitats, they would be unlikely to persist because of their poor competitiveness.

151. Where herbicides are used to control weeds in undisturbed environments glyphosate is frequently used, but removal is normally by spot spraying, not broadcast spraying, and if Roundup Ready® canola did occur in these environments it could be effectively controlled using other herbicides and non-chemical management techniques.

5.5.2 Weed risk assessment of parental GM canola lines

152. A weed risk assessment of non-GM canola based on the National Post-Border Weed Risk Management Protocol is included in the reference document *"The Biology of* Brassica napus L. *(canola)* (see Appendix 1, OGTR 2011) and summarised in Section 4.2.2 above. The genetic modifications in the GM parental lines do not alter the ratings for invasiveness or impact in any of the land uses where canola primarily occurs, namely, dryland and irrigated agricultural areas, and highly disturbed areas such as roadsides. The property of herbicide tolerance (either to glufosinate ammonium for InVigor® canola or to glyphosate for Roundup Ready® canola) could affect the plant's tolerance to average weed management practices. However, as discussed above, all of the parental GM canola lines remain susceptible to alternative herbicides, as well as standard agronomic and mechanical management practices.

153. These conclusions are consistent with the RARMPs prepared for DIR 020/2002 and DIR 021/2002, which assessed the risk of increased weediness from commercial release of these GMOs as negligible when compared to non-GM canola.

5.5.3 Herbicide resistance

154. There is some potential for development of herbicide-resistant weeds if the parental GM canola lines and their corresponding herbicides are used inappropriately. The repetitious use of a single herbicide, or herbicide group⁸, increases the likelihood of selecting weeds that have developed herbicide resistance through natural mechanisms (Gressel 2002). Integrated weed management practices help to avoid selection of resistant weed biotypes (CropLife Australia 2011).

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

156. The development of resistance to glufosinate ammonium and glyphosate herbicides would have implications for the choice of herbicide(s) available for weed control operations in agriculture and elsewhere. The APVMA assesses all herbicides used in Australia and sets their conditions of use.

157. Glyphosate has historically been considered a low risk herbicide for the development of herbicide resistance because its mode of action imposes genetic and biochemical constraints associated with potential mechanisms of resistance (Jasieniuk 1995; Bradshaw et al. 1997) and the frequency of mutations that impart glyphosate tolerance in plants is lower than for other herbicides (Weersink et al. 2005). However, the recent intensive use of glyphosate across large areas has resulted in several reports of glyphosate-tolerant weed

⁸ Herbicides are classified into groups based on their mode of action. All herbicide product labels must display the mode of action group. This enables users to rotate among herbicides with different modes of action to delay the development of herbicide tolerance in weeds.

species (Powles et al. 1998; Pratley et al. 1999; Neve et al. 2004; Powles & Preston 2006; Yu et al. 2006; Green et al. 2008).

158. Among others, these weeds include: *Lolium rigidum* (rigid ryegrass) in Australia; *Conyza bonariensis* (hairy fleabane) in South Africa and North America; *Eluesine indica* (goosegrass) in Malaysia; *Lolium multiflorum* (Italian ryegrass) in Chile; *Plantago lanceolata* (Buckhorn plantain) in South Africa; and *Cyperus esculentus* (yellow nutsedge), *Commelina benghalensis* (tropical spiderwort), *Ipomoea* spp. (morning glory) and *Acalypha* (wild buckwheat) in North America (Powles & Preston 2006; Green et al. 2008; Heap 2011).

159. A review in 2008 found no reports of glufosinate ammonium tolerant weeds (Green et al. 2008). Since then, there has only been one report of a glufosinate ammonium tolerant weed (*E. indica* in Malaysia in 2009, Heap 2011).

160. Stacking of multiple herbicide tolerant traits, such as in the InVigor® x Roundup Ready® canola proposed for release, increases the number of herbicide mixture options with multiple modes of action (Green et al. 2008). This could reduce the selective pressure on weed populations that occurs when a single herbicide is used exclusively.

161. Crop Management Plans have been developed separately by Bayer CropScience and Monsanto for InVigor® and Roundup Ready® canola, respectively. These CMPs are required to be followed by canola growers when growing either InVigor® canola, Roundup Ready® canola or InVigor® x Roundup Ready® canola. The CMPs address issues such as minimising and managing canola volunteers in crops following GM herbicide tolerant canola in a rotation, and minimising the development of herbicide tolerant weeds.

5.6 Potential for gene transfer from the parental GM canola lines

162. The potential for gene transfer from the parental GM canola lines to other sexually compatible plants (including other herbicide tolerant canola crops) was assessed in the RARMPs for licences DIR 020/2002 and 021/2002. A brief summary of this assessment, along with any new or updated information, is provided below.

163. Any transfer of the *barnase* gene to other sexually compatible plants will not have any negative environmental impacts because it will only result in male sterility and not confer any selective advantage in terms of weediness or persistence. The fertility restorer gene (*barstar*) would have no impact on a plant's phenotype apart from restoring male fertility for a portion of the progeny of a cross with a plant containing the male sterile gene. Therefore, only the potential for transfer of the herbicide tolerance traits is discussed below.

5.6.1 Gene transfer to other canola crops

164. Canola is predominantly self-pollinating with average inter-plant outcrossing rates of 30%. Outcrossing frequencies are highest in the first 10 m of the recipient fields, and rates decline with distance (Husken & Dietz-Pfeilstetter 2007). In a commercial situation, where different canola crops may be grown in adjacent fields, outcrossing is likely to occur beyond 10 m of the field borders. Cross pollination between canola lines is inevitable given sufficient proximity and exposure. There was no indication that the genetic modifications of the parental GM canola lines would increase the rate of outcrossing.

165. If Roundup Ready® or InVigor® canola is grown in close proximity to other canola crops there is a high likelihood of some outcrossing resulting in herbicide tolerant volunteers in adjacent fields where GM herbicide tolerant canola has not been grown. However, the overall frequency of hybridisation will be low and the number of resultant herbicide tolerant volunteers would be reduced by the vast majority of hybrid seeds being harvested along with

the crop. Such volunteers would pose the same negligible risk to human health and safety and the environment as the parental GM canola, as assessed in DIR 020/2002 and DIR021/2002.

166. The possibility of gene transfer from Roundup Ready® or InVigor® canola crops would make the management of canola volunteers more complex and have implications for the choice of herbicide(s) selected for control operations, not only for growers of GM herbicide tolerant canola, but also for growers of other canola varieties. However, as discussed previously, volunteers can be readily controlled by alternative herbicide and non-chemical management practices currently used to control canola volunteers.

Gene transfer to herbicide tolerant canola

167. The 'stacking' of multiple herbicide tolerance traits through outcrossing between the two GM herbicide tolerant canolas and non-GM herbicide tolerant canola varieties could also occur at a low frequency, and would have implications for herbicide choices for the control of canola volunteers. In 2005–2006, approximately 75% of the canola crop in Australia comprised non-GM imidazolinone tolerant (Clearfield®) and triazine tolerant (TT) varieties (Norton & Roush 2007).

168. Note that because the triazine tolerance trait in TT canola is maternally inherited, and so cannot be spread by pollen movement, stacking of the glyphosate or glufosinate ammonium tolerance traits will only occur in the direction of Roundup Ready® or InVigor® canola to TT canola, and not *vice versa*.

169. Hybridisation between the existing non-GM herbicide-tolerant canola varieties, InVigor® canola and Roundup Ready® canola could result in accumulation or 'stacking' of genes for tolerance to up to four different herbicide groups within the same plant. However, development of canola plants with all four herbicide tolerance traits would only be expected to occur at an extremely low frequency because it would require at least three separate hybridisation events (two crosses between different pairs of herbicide tolerant canolas and a cross between the progeny of these).

170. Attention to volunteer management, proper crop rotation and herbicide management practices should limit the frequency of productive hybridisation between different herbicide tolerant canola varieties and hence the development of multiple herbicide tolerant canola in Australia (Rieger et al. 2001; Downey 1999; Salisbury 2002c). If multiple-herbicide tolerant canola plants were to occur, they are unlikely to be more invasive or persistent than non-herbicide tolerant canola plants and could be controlled by other herbicides or other agricultural practices.

5.6.2 Gene transfer to other sexually compatible species

171. Canola can cross with other *B. napus* groups or subspecies (including vegetable forms), *B. oleracea, B. juncea* and *B. rapa* under natural conditions. Naturally occurring hybrids between *B. napus* and *R. raphanistrum, H. incana* and *S. arvensis* have also been reported at very low frequencies (Salisbury 2002b; Warwick et al. 2009). All of these species are naturalized in Australia and weedy forms are known to be present (Groves et al. 2003). *B. juncea, H. incana, R. raphanistrum* and *S. arvensis* are problematic weeds in commercial canola growing regions of Australia. Therefore, it is likely that some or all of these sexually compatible species may be found growing at or near sites where the parental GM canola lines are grown. Hybridisation requires synchronicity of flowering between the parental GM canola lines and sexually compatible species to enable cross-pollination and gene flow to occur.

172. The RARMPs prepared for DIR 020/2002 and 021/2002 assessed the risks associated with gene flow from the parental GM canola lines to *B. rapa, H. incana, R. raphanistrum* and

S. arvensis as very low, while the risks associated with gene flow to *B. napus* vegetables and forage rape, *B. oleracea* or *B. juncea* were assessed as negligible.

173. *B. napus* vegetables or forage are generally harvested or used for forage before flowering. *B. napus* vegetable seed production crops are isolated from other *B. napus* vegetable or canola crops to prevent outcrossing. Of the other sexually compatible *Brassica* species, hybridization offurs most readily between canola and *B. rapa*. Hybrids are often observed when the two species are grown in close proximity (Simard et al. 2006) and the transfer of traits from commercially grown canola to wild populations of *B. rapa* has been observed in Canada (Warwick et al. 2003). Warwick et al. (2008) showed that a herbicide tolerance trait from a commercial canola crop was transferred to, and stably maintained in, a wild *B. rapa* population for at least six years. The trait persisted despite the fact that the corresponding herbicide had not been applied during this period and, hence, no selective pressure had been applied.

174. The research of Warwick et al. (2008) illustrates that, if plants are growing in close proximity with synchronous or overlapping flowering periods, gene flow to sexually compatible species can occur. However, all interspecific hybrids have reduced fertility and low seed set due to the genetic barriers that exist (Jorgensen & Andersen 1994; Jorgensen et al. 1998; Salisbury 2002a; Warwick et al. 2003; Salisbury 2006). With the exception of the relatively productive interspecific hybridisation that occurs between *Brassica* species that contain the A genome (*B. napus*, *B. juncea* and *B. rapa*), most other interspecific hybridisation events occur at very low frequency.

175. Gene transfer from the parental GM canola lines to brassicaceous weeds would have implications for the choice of herbicide(s) for control of brassicaceous weeds. Glyphosate or glufosinate ammonium tolerant hybrids can be effectively controlled using a range of alternative herbicides and other non-chemical management techniques currently used for the control of Brassicaceous weeds. In addition, glyphosate or glufosinate ammonium would not be used for weed control in or adjacent to paddocks where Roundup Ready® or InVigor® canola has been grown because it would be ineffective in controlling the GM herbicide tolerant canola volunteers. Measures taken to control GM herbicide tolerant canola volunteers would also eliminate any herbicide tolerant hybrids.

Section 6 The GMO, nature and effect of the genetic modification

6.1 Introduction to the GMO

176. The InVigor® x Roundup Ready® canola that Bayer intends to commercialise in Australia in the current application is derived from conventional breeding between InVigor® canola lines MS8 and RF3 and Roundup Ready® canola line GT73.

177. The InVigor® x Roundup Ready® canola would most likely be produced by crossing a MS8 x GT73 hybrid with RF3, but Bayer may also produce it by crossing a RF3 x GT73 hybrid with MS8, or by crossing the two hybrids together (RF3 x GT73 and MS8 x GT73). Hybrids MS8 x GT73 and RF3 x GT73 would therefore be grown by Bayer for breeding and seed production purposes. In addition, Bayer has indicated that it may use the RF3 x GT73 hybrid for cropping in the future.

178. Although they are not intended for commercial release, Bayer is also seeking approval from the Regulator for release of GM canola hybrids derived from conventional breeding between GM Roundup Ready® line GT73 and the remaining GM canola lines authorised for release under licence DIR 021/2002 *i.e.* T45, Topas 19/2, MS1, RF1 and RF2.

179. Based on the conventional crosses, the introduced genes present in the GM canola hybrids proposed for release are listed in Table 4. The InVigor® x Roundup Ready® canola that Bayer intends to commercialise will contain the *barnase* and *barstar* genes that comprise a hybrid breeding system; two copies of the *bar* gene conferring tolerance to glufosinate ammonium; and the *cp4 epsps* and *goxv247* genes that confer tolerance to glyphosate.

GM canola	Hybrid breeding system	Glufosinate ammonium tolerance	Glyphosate tolerance	Antibiotic resistance
MS8 x RF3 x GT73	barnase and barstar	2 copies of bar	cp4 epsps and goxv247	-
Reauy® canola)				-
MS8 x GT73	barnase	bar	cp4 epsps and goxv247	-
RF3 x GT73	barstar (2 copies)	bar	cp4 epsps and goxv247	-
T45 x GT73	-	pat	cp4 epsps and goxv247	-
Topas 19/2 x GT73	-	pat (2 copies)	cp4 epsps and goxv247	nptll (2 copies)
MS1 x GT73	barnase	bar	cp4 epsps and goxv247	nptll
RF1 x GT73	barstar	bar	cp4 epsps and goxv247	nptll
RF2 x GT73	barstar	bar	cp4 epsps and goxv247	nptll

 Table 4:
 The introduced genes present in the GM canola hybrids proposed for release

6.2 Characterisation of the GMO

180. Extensive data characterising the parental GM canola lines were provided with licence applications DIR 020/2002 and 021/2002. A brief summary of these analyses is provided below, with details available in the RARMPs prepared for these applications. In addition, Bayer has provided five reports characterising the InVigor® x Roundup Ready® canola proposed for commercial release. These reports are described in detail below.

6.2.1 Stability and molecular characterisation

Parental GM canola lines

181. Molecular characterisation of the parental GM canola lines included Southern blot and PCR analyses, as well as molecular cloning and sequencing of the site of insertion. Stable integration and inheritance of the inserted DNA was demonstrated in all of the lines. DNA sequencing was used to verify the inserted genes and to determine the regions flanking all of the insertions sites.

182. In lines T45, MS1, MS8, RF1, RF2 and GT73, a single insertion event occurred resulting in transfer of a single copy of the T-DNA. In line RF3, a single insertion event occurred that resulted in the integration of one complete copy and a second, incomplete T-DNA copy that included a second copy of the *barstar* gene. In line Topas 19/2, there is a single insertion event that resulted in a head to head inverted repeat of the T-DNA, such that there are two copies of each of the inserted *pat* and *nptII* genes.

183. Field trials of InVigor® canola and Roundup Ready® canola began in Australia in 1996 and 1997, respectively. Roundup Ready® canola has been grown commercially in NSW and Victoria since 2008, and in WA since 2010. In addition, events MS8, RF3 and GT73 have been commercialised for more than 10 years in Canada. In the multiple breeding programs and seed production, there have been no reports of aberrant segregation and instability.

InVigor® x Roundup Ready® canola

184. Southern blot analysis was used to demonstrate the molecular equivalence of the MS8, RF3 and GT73 events in InVigor® x Roundup Ready® canola to the same events in the individual parental lines. Identical Southern hybridisation patterns were observed for InVigor® x Roundup Ready® canola compared to InVigor® canola lines MS8 and RF3 and

to Roundup Ready® canola GT73. These findings confirm the intactness of the GM loci and their flanking regions in InVigor® x Roundup Ready® canola, indicating that no rearrangement occurred during conventional breeding (Moens 2009b).

6.2.2 Expression of the encoded proteins in the GM canola

Parental GM canola lines

185. The expression of each of the introduced genes in each of the GM canola lines authorised under DIR 021/2002 (Topas 19/2, T45, RF1, RF2, RF3, MS1 and MS8) was determined using a variety of techniques including plant phenotype, mRNA expression, and detection of the novel protein by enzyme activity or Enzyme Linked ImmunoSorbent Assays (ELISA). The patterns and levels of expression of the introduced proteins in the GM canola lines were as predicted on the basis of the promoters controlling expression, and a summary of these data is given in Table 5.

Table 5Summary of expression of the introduced proteins in GM canola lines included in
licence DIR 021/2002

Introduced Protein (GM lines assayed)	Leaves	Seed	Other tissues
PAT (All lines)	Low levels	Very low levels	Very low levels
BARNASE (MS1, MS8 or MS x RF)	Not expressed	Not expressed	Flower buds only: tapetum layer of developing anthers
BARSTAR (RF1, RF2, RF3 or MS x RF)	Not expressed	Not expressed	Flower buds only: tapetum layer of developing anthers
NPTII (Topas 19/2, RF1, RF2, MS1)	Very low levels	Not detected	Not detected

186. The level of PAT in oil and meal derived from processing of seed from lines T45 and Topas 19/2 was investigated by ELISA. No PAT protein was detected in canola oil derived from the GM canola lines. While PAT protein could be detected by ELISA at less than 0.005% of total protein in toasted canola meal, the processing of canola seed to produce edible oil and meal for animal feed denatures the PAT protein and destroys the enzymatic activity (FDA 1997; ANZFA 2001b).

187. Expression of the *bar, barnase, barstar, nptII* genes was also investigated by Northern analysis. Expression patterns were as predicted for the promoters used, and no mRNA from any of the genes was detected in pollen or dry seed.

188. The levels of expression of the CP4 EPSPS and GOXv247 proteins in leaf tissue and seeds of the parental Roundup Ready® canola were measured by ELISA (see DIR 020/2002). Results from several field trials conducted overseas demonstrate that the CP4 EPSPS and GOXv247 proteins are expressed at very low levels in leaves and seeds. The level of expression of CP4 EPSPS and GOXv247 constitutes less than 0.02% and 0.07%, respectively, of the seed on a fresh weight basis. Expression levels of the introduced proteins in Roundup Ready® canola were not affected by application of glyphosate.

InVigor[®] x Roundup Ready[®] canola

189. The expression levels of PAT, CP4 EPSPS and GOX proteins in leaf and seed tissues of InVigor® x Roundup Ready® canola and its parental lines MS8, RF3 and GT73 were measured by ELISA. Prior to sampling, MS8 and RF3 plants were treated with glufosinate ammonium, GT73 plants were treated with glyphosate, and MS8 x RF3 x GT73 plants were treated with both herbicides.

190. Table 6 provides a summary of the zygosity of the herbicide tolerance genes in the GM canola plants analysed in this study. GM canola line MS8 is hemizygous for the *bar* gene. Due to segregation, only 44% of the MS8 x RF3 x GT73 plants generated contained a copy of the *bar* gene from MS8. However, only MS8 x RF3 x GT73 plants containing the MS8 *bar* gene were used in this study.

Event/stack	<i>bar</i> gene	CP4 EPSPS gene	gox gene
MS8	hemizygous (1 copy)	-	-
RF3	homozygous (2 copies)	-	-
GT73	-	homozygous (2 copies)	homozygous (2 copies)
MS8 x RF3 x GT73	hemizygous for both MS8 and RF3 (2 copies)	hemizygous (1 copy)	hemizygous (1 copy)

 Table 6
 Zygosity of the herbicide tolerance genes in the GM canola plants

191. For each protein to be analysed, 10 separate leaf or seed samples were assayed. The average expression levels of the PAT, CP4 EPSPS and GOX proteins in samples of InVigor® x Roundup Ready® canola and the parent lines are given in Table 7. Differences in protein expression levels between the parent lines and the MS8 x RF3 x GT73 stack correlated with the zygosity of the plants. Protein expression levels in InVigor® x Roundup Ready® canola are either similar to or lower than the low levels observed in the parental lines. This analysis showed no evidence for any interaction between the three events when combined in InVigor® x Roundup Ready® canola (Moens 2009a).

Table 7Average amount of protein per gram fresh weight in leaf and seed tissue samples
from GM canola plants

Tissue	Line/stack	Average amount PAT (μg/g fresh weight ± SD)	Average amount CP4 EPSPS (µg/g fresh weight ± SD)	Average amount GOXv247 (μg/g fresh weight ± SD)
Leaf	MS8	10.0 ± 1.5	N/A	N/A
	RF3	22.6 ± 5.2	N/A	N/A
	GT73	N/A	72 ± 15	13.7 ± 3.1
	MS8 x RF3 x GT73	20.4 ± 4.8	51.7 ± 6.8	3.21 ± 0.89
Seed	MS8	2.63 ± 0.18	N/A	N/A
	RF3	5.09 ± 0.42	N/A	N/A
	GT73	N/A	112 ± 14	12.7 ± 1.7
	MS8 x RF3 x GT73	5.08 ± 0.30	62.8 ± 8.3	10.5 ± 1.4

SD = standard deviation; NA = not applicable

6.2.3 Agronomic characterisation

Parental GM canola lines

192. The growth characteristics of all of the parental GM canola lines were described in the RARMPs prepared for DIR 020/2002 and 021/2002. The parental GM canola lines do not differ from non-GM canola in flowering period; pollen production and pollen viability (except in the male sterile lines); seed production; seed shattering; seed size; seed weight; seed germination; seed dormancy; or agronomic performance, including disease susceptibility and sensitivity to herbicides other than glyphosate (for Roundup Ready® canola) or glufosinate ammonium (for GM canola lines Topas 19/2, T45, MS1, MS8, RF1, RF2 and RF3).

InVigor® x Roundup Ready® canola

193. The agronomic characteristics of InVigor® x Roundup Ready® canola were assessed during a field trial conducted in Canada during the 2008 growing season (Darragh & Rouan 2009). Harvested seed from this trial was also sent for nutritional analysis (see Section 6.2.4). The trial occurred at five locations in typical canola production regions of Canada that represented a range of environmental conditions and pest and disease pressures.

194. A randomised block design was used, with four repetitions per location. Within each of the five sites, ten categories of GM canola (Entries) with different hybrid backgrounds were grown (see Table 8). All hybrid backgrounds are commercially available in Canada. Plants were either treated with glufosinate ammonium and/or glyphosate, or were not treated with either of these herbicides, as described in Table 8.

Entry	GMO	Hybrid background	Herbicide treatment
number			
1	MS8 x RF3	А	glufosinate ammonium
2	MS8 x RF3	В	glufosinate ammonium
3	MS8 x RF3	С	glufosinate ammonium
4	MS8 x RF3 x	А	glyphosate + glufosinate ammonium
	GT73		
5	MS8 x RF3 x	A	not treated
	GT73		
6	MS8 x RF3 x	В	glyphosate + glufosinate ammonium
	GT73		
7	MS8 x RF3 x	В	not treated
	GT73		
8	GT73	D	glyphosate
9	GT73	E	glyphosate
10	GT73	F	glyphosate

 Table 8
 Description of the field trial design for agronomic characterisation of the GM canola

195. The plants were cultivated under typical agronomic practices for growing canola in Canada, including the use of conventional herbicides (not glyphosate or glufosinate ammonium), insecticides and fungicides as necessary.

196. The agronomic characteristics evaluated included:

- i. Agronomic performance: establishment, vigour pre-herbicide treatment, vigour 1 7 days post-herbicide treatment, vigour 15 – 20 days post-herbicide treatment, days to start and end of flowering, plant height, days to maturity, pod shattering, yield, germination and vigour of harvested grain.
- ii. Tolerance to biotic factors (insects and diseases)
- iii. Tolerance to heat stress

197. Overall, the agronomic characteristics of MS8 x RF3 x GT73 hybrids were comparable to their commercial MS8 x RF3 counterparts, apart from a small delay to maturity (less than one day; see below).

Effect of herbicide treatment

198. The effect of herbicide treatment was analysed by comparing herbicide treated and untreated MS8 x RF3 x GT73 plants (Entry 4 versus 5 and Entry 6 versus 7). No consistent effect of the herbicide treatment was observed in either hybrid background A or hybrid background B on the following characteristics: establishment; plant vigour; end of flowering; days to maturity; pod shattering; yield; grain germination; and grain vigour.

199. The untreated plants did start flowering significantly earlier, but the actual difference was only half a day or less. In hybrid background B, untreated plants were significantly shorter than treated plants, but this difference was not confirmed in hybrid background A.

MS8 x RF3 x GT73 versus MS8 x RF3 in comparable hybrid backgrounds

200. Statistical analysis was used to compare the agronomic performance of MS8 x RF3 plants to MS8 x RF3 x GT73 plants in hybrid backgrounds A and B (Entry 1 versus 4 and

Entry 2 versus 6). No overall significant differences were observed for establishment; days to flowering; pod shattering; yield, grain germination; or grain vigour.

201. At two sites, MS8 x RF3 x GT73 plants showed increased vigour compared to MS8 x RF3 plants before being herbicide treated. This difference continued on to 7 - 10 days post-spraying for one of the sites, but there were no differences in plant vigour 15 - 20 days post herbicide spray at any site.

202. There were no differences in agronomic characteristics that were consistent in both hybrid backgrounds. In hybrid background A, MS8 x RF3 x GT73 matured almost a day later than MS8 x RF3 plants, but no other significant differences were observed. In hybrid background B, MS8 x RF3 x GT73 plants matured about half a day earlier than MS8 x RF3 plants and flowering ended about 0.6 days sooner. MS8 x RF3 x GT73 plants were also about 5 cm shorter than MS8 x RF3 plants in hybrid background B.

MS8 x RF3 x GT73 versus other hybrid backgrounds

203. Descriptive statistics were used to make further comparisons between MS8 x RF3 x GT73 plants in hybrid backgrounds A and B and additional, distinct varieties carrying MS8 x RF3 (Entry 3) or GT73 (Entries 8, 9 and 10). Overall, minimum and maximum values reported for MS8 x RF3 x GT73 plants were well within the range of values reported for the commercial hybrids. Only the yield seemed to be slightly higher in the MS8 x RF3 x GT73 plants were compared to MS8 x RF3 plants in the same hybrid backgrounds (A and B, as described above), statistical analyses showed that the small differences were not significant.

Biotic and abiotic stress

204. Insect damage, disease symptoms and heat stress symptoms were observed over the course of the experiment. The presence of common insects or diseases was noted on regular site visits. If present, all Entries were given a score between 1 and 9 as an indication of plant health. The following common insects and diseases were observed during the trial:

- Insects flea beetles, diamond black moth, lygus bugs, bertha armyworm, aphids and cabbage seed pod weevil.
- Diseases blackleg, sclerotinia, alternaria black spot, fusarium wilt, downy mildew and white rust.

205. Some variation in insect damage was observed between sites, however different sites were subject to different pesticide spray regimes. Within each site, no differences in insect damage, disease symptoms or heat stress symptoms were observed for different plants or different treatments. However, this qualitative analysis would only pick up gross differences in stress symptoms.

6.2.4 Compositional analyses

Parental GM canola lines

206. Compositional analyses were provided for each of the parent lines used to generate the GM canola proposed for release (Topas 19/2, T45, MS1, MS8, RF1, RF2, RF3 and GT73), as well as for InVigor® Hybrid canola (MS8 x RF3). Details of these analyses are available in the RARMPs for DIRs 020/2002 and 021/2002.

207. In summary, the levels of erucic acid and glucosinolates in all parental GM canola lines are below the industry standards and do not vary significantly from their parental cultivars or other commercially available canola.

208. Compositional analyses demonstrate that the parental GM canola lines, and the MS8 x RF3 hybrid, are comparable in composition (including fatty acid content, protein content and proximate analyses) to their parental non-GM cultivars, and to other commercial canola cultivars when grown at a variety of different locations, including Canada, Europe and Australia.

209. Application of the herbicides glufosinate ammonium on the InVigor® canola lines and glyphosate on Roundup Ready® canola did not have a significant effect on any of the compositional parameters investigated.

InVigor® x Roundup Ready® canola

210. Bayer has provided two nutritional impact assessment reports for InVigor® x Roundup Ready® canola (Oberdörfer 2011a; Oberdörfer 2011b). Both reports analysed the same seed components in different GM and non-GM canola hybrids: proximate and fibre compounds, minerals, tocopherols, amino acids, fatty acids, and the anti-nutrients phytic acid, glucosinolates and erucic acid.

211. In summary, the reports demonstrate that MS8 x RF3 x GT73 hybrid canola is nutritionally equivalent to commercial MS8 x RF3 canola and to other commercial canola hybrids. There is no impact on the nutritional value of MS8 x RF3 x GT73 hybrid canola as a result of herbicide treatment, the genetic modifications, or combining the glufosinate ammonium tolerance trait with the glyphosate tolerance trait by conventional breeding. Small differences were detected for some components (including some glucosinolates) but the mean values for these compounds are within the ranges calculated for commercial canola hybrids grown in the same trials, and in good agreement with ranges available in published literature.

212. The levels of glucosinoloates and erucic acid in InVigor® x Roundup Ready® canola are within the range observed in MS8 x RF3 and other commercial hybrids, and are well below the standard thresholds (2% erucic acid in the oil and 30 μ moles g⁻¹ glucosinolates in the meal).

Report 1 : MS8 x RF3 x GT73 versus MS8 x RF3

213. The first report used seed collected from the field trial described in Section 6.2.3 to compare MS8 x RF3 x GT73 hybrids to their commercial MS8 x RF3 counterparts (Oberdörfer 2011b). Statistical analyses were done separately for hybrid background A and hybrid background B Entries. The impact of herbicide treatment on MS8 x RF3 x GT73 hybrids was evaluated by comparing seed from treated and untreated MS8 x RF3 x GT73 plants (Entry 4 versus 5 and Entry 6 versus 7). The impact of stacking InVigor® canola with Roundup Ready® canola was evaluated by comparing MS8 x RF3 to MS8 x RF3 x GT73 seeds (Entry 1 versus 4 and Entry 2 versus 6).

214. For most compounds (48/58 in hybrid background A and 47/58 in hybrid background B), there were no significant differences between the Entries over all sites and in both hybrid backgrounds. However, statistically significant Entry effects were detected for a few components, as discussed below.

215. In hybrid background A, significant differences were detected between seeds of herbicide treated MS8 x RF3 and MS8 x RF3 x GT73 (Entries 1 and 4) for phosphorous, zinc, delta tocopherol, alkenyl glucosinolate, total glucosinolate, stearic acid (C18:0), arachidic acid (C20:0), eicosadienoic acid (C20:2) and behenic acid (C22:0). A significant difference was also detected between seeds of herbicide treated and untreated MS8 x RF3 x GT73 (Entries 4 and 5) for myristic acid (C14:0) (see Table 9).

216. In hybrid background B, significant differences were detected between herbicide treated MS8 x RF3 and MS8 x RF3 x GT73 seeds (Entries 2 and 6) for moisture, delta tocopherol, alkenyl glucosinolates, MSGL glucosinolates, stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosadienoic acid (C20:2) and lignoceric acid (C24:0) (see Table 10).

217. To evaluate the biological and nutritional relevance of these differences, the average values were compared to reference ranges from four commercial canola hybrids (Entries 3, 8, 9 and 10) as well as from published references (Table 9 and Table 10). The comparison with the commercial hybrids is most relevant as the plants were all grown at the same sites and the same methods were used for the analyses. For all of the components that showed a significant Entry effect, the absolute differences are small, and the mean values are within the reference ranges calculated from the tested commercial canola hybrids, and in good agreement with those available from published references. This suggests that there is no major effect on the content of the nutrients caused by the herbicide treatment of MS8 x RF3 x GT73, and that MS8 x RF3 x GT73 hybrid canola is compositionally similar to MS8 x RF3 commercial canola in the same hybrid background.

Table 9	Values for compounds in seeds of MS8 x RF3 x GT73 and MS8 x RF3 in hybrid
	background A compared to ranges from other canola hybrid backgrounds and from
	published data. Only compounds that varied between Entries are shown.

	Entry 1 MS8 x RF3 herbicide treated ^a	Entry 4 MS8 x RF3 x GT73 herbicide ^a treated	Entry 5 MS8 x RF3 x GT73 not treated ^a	Range from Entries 3, 8, 9, 10 (other canola hybrids)	Range from published data
phosphorous ^c	6490 ± 570	6240 ± 430	6380 ± 440	5290 – 8110	4800 – 8500 ^f
zinc °	47.8 ± 4.3	46.2 ± 4.9	46.3 ± 4.4	32.8 – 58.8	62 ^f
iron °	64.8 ± 12.6	60.1 ± 7.5	75.9 ± 37.8	50 - 249	Not available
delta tocopherol °	7.22 ± 1.9	7.80 ± 1.9	7.78 ± 1.8	< 5.00 – 10.5	0 – 12 ^{g, h}
alkenyl glucosinolates d	5.17 ± 1.37	4.43 ± 1.09	4.87 ± 1.79	2.93 – 14.56	Not available
total glucosinolates ^d	10.81 ± 1.53	9.75 ± 1.10	9.63 ± 1.60	6.86 – 20.55	6 – 29 (in meal) ^f
stearic acid ^e (C18:0)	2.21 ± 0.21	2.30 ± 0.79	2.30 ± 0.19	1.58 – 2.59	0.8 – 3.0 ^g
arachidic acid ^e (C20:0)	0.73 ± 0.05	0.76 ± 0.05	0.75 ± 0.06	0.55 – 0.85	0.2 – 1.2 º
eicosadienoic acid e (C20:2)	0.062 ± 0.01	0.058 ± 0.01	0.059 ± 0.01	<0.01 – 0.07	0 – 0.1 ^g
behenic acid ^e C22:0	0.38 ± 0.03	0.39 ± 0.03	0.39 ± 0.03	0.24 – 0.44	0 – 0.6 g

See key for Table 10

Table 10Values for compounds in seeds of MS8 x RF3 x GT73 and MS8 x RF3 in hybrid
background B compared to ranges from other canola hybrid backgrounds and from
published data. Only compounds that varied between Entries are shown.

	Entry 2 MS8 x RF3 herbicide treated ^a	Entry 6 MS8 x RF3 x GT73 herbicide treated ^a	Entry 7 MS8 x RF3 x GT73 not treated ^a	Range from Entries 3, 8, 9, 10 (other canola hybrids)	Range from published data
moisture ^b	5.90 ± 0.35	6.07 ± 0.25	6.06 ± 0.21	4.97 – 6.51	7.4 – 10 ^f
delta tocopherol ^c	7.05 ± 1.3	7.55 ± 1.22	7.76 ± 1.5	<5.00 – 10.5	0 – 12 ^{g, h}
alkenyl glucosinolates d	6.12 ± 1.77	5.09 ± 1.37	5.12 ± 1.5	2.93 – 14.56	Not available

	Entry 2 MS8 x RF3 herbicide treated ^a	Entry 6 MS8 x RF3 x GT73 herbicide treated ^a	Entry 7 MS8 x RF3 x GT73 not treated ^a	Range from Entries 3, 8, 9, 10 (other canola hybrids)	Range from published data
MSGL glucosinolates d	0.11 ± 0.08	0.060 ± 0.03	0.082 ± 0.06	<0.05 – 0.41	Not available
stearic acid ^e (C18:0)	2.04 ± 0.20	2.15 ± 0.19	2.14 ± 0.19	1.58 – 2.59	0.8 – 3.0 ^g
oleic acid ^e (C18:1)	62.44 ± 1.47	63.51 ± 1.10	63.25 ± 1.36	61.28 – 66.46	51.0 – 70.0 g
linoleic acid ^e (C18:2)	17.48 ± 0.50	16.70 ± 0.43	16.87 ± 0.31	15.89 – 19.37	15.0 – 30.0 ^g
linolenic acid ^e (C18:3)	10.47 1.29	10.05 ± 1.08	10.09 ± 1.34	7.27 – 11.13	5.0 – 14 g
arachidic acid ^e (C20:0)	0.68 ± 0.04	0.72 ± 0.05	0.72 ± 0.04	0.55 – 0.85	0.2 – 1.2 ^g
eicosadienoic acid ^e (C20:2)	0.070 ± 0.004	0.064 ± 0.005	0.062 ± 0.004	<0.01 – 0.07	0 – 0.1 g
lignoceric acid e	0.22 ± 0.02	0.23 ± 0.03	0.24 ± 0.03	0.13 – 0.31	0 – 0.3 g

a: Mean ± SD; b: %; c: mg/kg; d: µmol/g; e: % of total fatty acids; f: OECD (2001); g: CODEX (2009); h: Converted from mg/kg crude oil to mg/kg dry matter in seed based on a seed fat content of 24.0 – 52.6% dm

Report 2 : MS8 x RF3 x GT73 versus a non-GM comparator

218. In the second report, MS8 x RF3 x GT73 hybrids are compared to a non-GM canola comparator in the same hybrid background and to other canola hybrids commercially available in Canada (Oberdörfer 2011a). The canola was grown at four field trial sites in Canada in 2010. At each site, six categories of canola (Entries) were planted on three replicate plots arranged in a randomised block design. The Entries and their treatments are described in Table 11.

 Table 11: Description of the field trial design for composition analysis of GM and non-GM canola

Entry	Canola	Treatment
11	MS8 x RF3 x GT73 (hybrid background A)	glyphosate and glufosinate ammonium
12	MS8 x RF3 x GT73 (hybrid background A)	not treated
13	non-GM comparator (hybrid background A)	not treated
14	commercial variety hybrid 1	glyphosate
15	commercial variety hybrid 2	glufosinate ammonium
16	commercial variety hybrid 3	glufosinate ammonium

219. Statistical analyses comparing the composition data from Entries 11 - 13 showed no significant Entry effect for most compounds (48/58). However, significant Entry effects were again detected for a few components, as discussed below.

220. A significant difference in moisture was detected between MS8 x RF3 x GT73 canola treated with glyphosate and glufosinate ammonium (Entry 11) and the non-GM comparator (Entry 13). However, no difference was detected between untreated MS8 x RF3 x GT73 canola (Entry 12) and the non-GM comparator (Entry 13). The analysis was repeated on a site-by-site basis, which showed no significant differences between the Entries at any of the sites.

221. Significant differences between MS8 x RF3 x GT73 canola, both treated and untreated, and the non-GM comparator (Entry 11 vs. 13 and Entry 12 vs. 13) were detected for alpha tocopherol; alkenyl glucosinolates; the minerals calcium and manganese; and five fatty acids (C18:2 linoleic acid, C18:3 linoleic acid, C22:0 behenic acid, C24:0 lignoceric acid and C24:1 nervonic acid). However, for all of the compounds which showed a significant

Entry effect, the absolute differences are small, and the mean values are within the reference ranges from the three commercial canola hybrids (Entries 14 - 16) and in good agreement with those available from published references (Table 12). This suggests that MS8 x RF3 x GT73 hybrid canola is compositionally similar to non-GM canola in the same hybrid background.

Table 12	Values for compounds in seeds of MS8 x RF3 x GT73 canola and the non-GM
	comparator compared to ranges from commercial canola hybrids and from
	published literature. Only compounds that varied between Entries are shown.

	Entry 11 (MS8 x RF3 x GT73 herbicide treated) ^a	Entry 12 (MS8 x RF3 x GT73 not treated) ^a	Entry 13 non-GM comparator ^a	Range from Entries 14-16 (commercial hybrids)	Range from published data
calcium ^b	3968 ± 361	3942 ± 563	3478 ± 549	3110 – 5310	2900 – 4800 f
manganese ^b	33.4 ± 4.7	34.2 ± 5.5	37.6 ± 4.6	20.6 – 40.2	Not available
alpha tocopherol ^b	76.0 ± 16.9	73.1 ± 17.9	89.7 ± 20.9	37.4 – 125.0	24 – 203 ^{e, g}
alkenyl glucosinolates °	6.47 ± 1.90	6.23 ± 1.53	7.37 ± 0.95	2.34 – 8.06	Not available
total glucosinolates c	13.02 ± 2.54	12.58 ± 2.24	13.62 ± 1.75	6.38 – 14.26	6 – 29 ^f (in meal)
linoleic acid ^d C18:2	18.33 ± 0.89	18.21 ± 0.85	19.35 ± 1.04	17.81 – 23.21	15.0 – 30.0 ^g
linoleic acid ^d C18:3	12.21 ± 1.26	12.15 ± 1.26	11.22 ± 1.41	9.32 – 18.83	5.0 – 14.0 º
behenic acid ^d C22:0	0.414 ± 0.043	0.415 ± 0.043	0.435 ± 0.048	0.270 – 0.44	0 – 0.6 ^g
lignoceric acid d C24:0	0.236 ± 0.035	0.228 ± 0.035	0.211 ± 0.039	0.110 – 0.320	0 – 0.3 g
nervonic acid d C24:1	0.278 ± 0.073	0.280 ± 0.068	0.246 ± 0.067	0.150 – 0.340	0 - 0.4 g

a; Mean \pm SD; b: mg/kg dry matter in seed; c: μ mol/g dry matter in seed; d: % of total fatty acids; e: Converted from mg/kg crude oil to mg/kg dry matter in seed based on a seed fat content of 24.0 – 52.6% dm; f: OECD (2001); g: CODEX (2009)

Section 7 The receiving environment

222. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes: any relevant biotic/abiotic properties of the geographic regions where the release would occur; intended agricultural practices, including those that may be altered in relation to normal practices; other relevant GMOs already released; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release (OGTR 2009b).

223. The applicant has proposed to release InVigor® x Roundup Ready® canola in all commercial canola growing areas of Australia. Suitable areas for canola cultivation may change over time. Therefore, for this particular licence application, it is considered that the receiving environment would be Australia-wide. The initial GM varieties proposed for release will be suited to areas where there is high rainfall and medium to long season. Further varieties will be developed which are suited to areas of lower rainfall and other season lengths.

224. The applicant proposes that all plant materials and derived products would be allowed to enter general commerce, including use in human food and animal feed, such that GM plant material may be transported and used Australia-wide.

7.1 Relevant abiotic factors

225. The abiotic factors relevant to the growth and distribution of canola currently used in commercial production in Australia are discussed in *The Biology of* Brassica napus *L.* (canola) document (OGTR 2011). In brief, the geographical distribution of commercial canola cultivation in Australia is limited by a number of abiotic factors, the most important being water availability.

226. Canola is generally grown as a winter crop in dominant winter rainfall environments that receive > 400 mm rainfall per year. Sufficient soil moisture is required for germination of seed, and drought stress after anthesis can significantly reduce yield due to abortion of seed and reduced pod numbers. However, canola is also sensitive to waterlogged soils, so sites prone to water-logging tend to be avoided by commercial producers (Walton et al. 1999). Canola can also be grown during summer, but only at sites that receive sufficient rainfall or are under irrigation. For this reason, summer cultivation is generally restricted to high-value seed production.

227. Soil nutrient availability is also an important abiotic factor affecting canola cultivation. Most Australian soils tend to be low in nutrients and canola can only be profitably grown if fertilisers are intensively applied (Hocking et al. 1999). Other abiotic factors that can reduce seed yields include high soil acidity, frost and high temperatures.

228. Additional information regarding the abiotic factors relevant to the growth and distribution of commercial canola in Australia are discussed in *The Biology of* Brassica napus *L.(canola)* (OGTR 2011).

7.2 Relevant biotic factors

7.2.1 Presence of related plants in the receiving environment

229. Commercial canola varieties grown in Australia include non-GM varieties that are susceptible to herbicides, as well as non-GM and GM herbicide tolerant varieties.

230. Weeds are a major factor limiting commercial canola production in Australia and the importance of effective weed control to growers is exemplified by the fact that approximately 75% of canola grown in Australia in 2005-6 was herbicide tolerant (Norton & Roush 2007). There are two conventionally bred herbicide tolerant canola varieties currently being grown throughout Australia – triazine tolerant and imidazolinone tolerant. Since the introduction of non-GM triazine tolerant canola varieties in 1993 their use has become widespread despite a significant yield penalty associated with the mutation that confers herbicide tolerance. The first non-GM imidazolinone tolerant canola variety was registered for use in 1995, and together triazine and imidazolinone tolerant varieties comprise approximately 75% of the Australian canola crop (Norton & Roush 2007).

231. GM Roundup Ready® canola was approved for unrestricted commercial release by the Regulator in 2003 (DIR 020/2002). However, it was not grown commercially until 2008 (New South Wales and Victoria) and 2010 (Western Australia), due to restrictions imposed by State and Territory governments for marketing and trade reasons. InVigor® canola was also approved for commercial release by the Regulator in 2003 (DIR 021/2002), but has not yet entered commercial production. In the 2010 growing season, around 8% of canola grown in Australia was GM, most of which (50 - 60%) is grown in WA (DAFWA 2010). Therefore, there are currently three herbicide tolerance traits present in commercial production systems, and potentially a fourth in the future, that could inadvertently combine with each other. The hybrid GM canola proposed for release by Bayer could also potentially combine with the non-GM herbicide tolerant canola to produce multiple-herbicide tolerant progeny.

232. *B. napus* is known to cross with other species within the *Brassicaceae* tribe. Of the many *Brassica* species in Australia, canola may potentially hybridise under natural conditions with sexually compatible related species that include: other *B. napus* groups or subspecies (including vegetables such as Swedes, rutabaga, kale), *B. juncea* (Indian mustard), *B. rapa* (canola, turnip rape or white turnip; includes vegetables such as turnip, chinese cabbage and pak choi) and *B. oleracea* (wild cabbage; includes vegetables such as cauliflower, brussel sprouts and cabbage) (Salisbury 2002b). Naturally occurring hybrids between *B. napus* and species from other genera in the *Brassicaceae* tribe have been reported at very low frequencies for *Raphanus raphanistrum* (wild radish), *Hirschfeldia incana* (Buchan weed) and *Sinapis arvensis* (charlock) (Salisbury 2002b) (see Section 5.6.2 for more detail).

7.2.2 Presence of other biotic factors

233. A number of diseases have potential to significantly reduce the yield of canola. Blackleg disease caused by the fungal pathogen *Leptosphaeria maculans* is the most devastating disease affecting commercial canola production in Australia. Other diseases of canola include *Sclerotinia* stem rot, *Rhizoctonia* seedling wilt and *Alternaria* black spot, all of which are caused by fungal pathogens (Howlett et al. 1999).

234. 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 during flowering and podding (Miles & McDonald 1999; Oilseeds WA 2006).

235. Weeds are also a significant problem for commercial canola producers and can reduce yield by competition and seed quality due to contamination. The most significant weeds include annual ryegrass, members of the fescue genus, volunteer cereals and a large number of *Brassicaceous* weeds. The most detrimental *Brassicaceous* weeds are wild radish (*Raphanus raphinastrum*), Indian hedgemustard (*Sisymbrium orientale*), Shepherd's purse (*Capsella bursa-pastoris*), wild turnip (*Brassica tournefortii*), turnip weed (*R. rugosum*), charlock (*Sinapis arvensis*), musk weed (*Myagrum perfoliatum*) and Buchan weed (*Hirschfeldia incana*) (Sutherland 1999), some of which are sexually compatible with canola, as described in Sections 5.6.2and 7.2.1.

236. Additional information regarding the biotic factors relating to the growth and distribution of commercial canola in Australia are discussed in the reference document, *The Biology of* Brassica napus *L. (canola)* (OGTR 2011).

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

237. The introduced genes and regulatory sequences were originally isolated from naturally occurring organisms, which are already widespread and prevalent in the environment.

238. The bacterium *B. amyloliquefaciens*, from which the *barnase* and *barstar* genes were obtained, is a commonly occurring soil bacterium that is widespread in nature and is frequently used in industry (see Section 5.1.3) (ANZFA 2001b). BARNASE is a ribonuclease enzyme that is secreted by *B. amyloliquefaciens* into the soil and BARSTAR is a ribonuclease inhibitor protein which specifically inhibits BARNASE enzyme function. Ribonuclease enzymes and ribonuclease inhibitor proteins are ubiquitous in nature and can be found in plants, animals and microorganisms. Therefore, both the source organism (*B. amyloliquefaciens*) and the classes of protein encoded by the introduced genes (ribonuclease and ribonuclease inhibitor) would be commonly encountered by other organisms in the environment.

239. The introduced *cp4 epsps* gene was isolated from the common soil bacterium *A. tumefaciens*. Homologues of *cp4 epsps* and its encoded enzyme occur naturally in all plants, bacteria and fungi, including plants widely consumed by animals and people, and in some microoganisms which are plant pathogens (Kamada-Nobusada & Sakakibara 2009).

240. The *goxv247* gene is derived from *O. anthropi* strain LBAA, a bacterium commonly found in the soil. The *goxv247* gene encodes the GOXv247 protein that differs from the native *O. anthropi* enzyme by three amino acids.

241. PAT proteins are produced naturally by the common soil bacteria

S. viridochromogenes and *S. hygroscopicus*, encoded by the *pat* and *bar* genes, respectively (Wohlleben et al. 1988; Strauch et al. 1988). These species of *Streptomyces* are common soil dwelling bacteria (Lawrence 2000), which can naturally develop the ability to detoxify glufosinate ammonium (Bartsch & Tebbe 1989). Genes encoding PAT or similar enzymes are present in a wide variety of bacteria. Acetyltransferases, the class of enzymes to which PAT belongs, are common enzymes in all microorganisms, plants and animals. Different versions of PAT protein have also been expressed in other GM crop plants trialled in Australia (DIRs 010/2001, 015/2002, 016/2002, 036/2003, 038/2003, 040/2003 and 044/2003) or commercially approved (canola DIR 021/2003, cotton DIR 062/2005 and cotton DIR 091).

242. Short regulatory sequences necessary to control expression of the novel genes have been derived from: the common soil bacterium *A. tumefaciens*; the plant species *A. thaliana* (thale cress), *N. tabacum* (tobacco) and *P. sativum* (pea); and the plant viral pathogens CaMV and FMV. These organisms are all widespread in the environment. Although some of these sequences are derived from plant pathogens (*A. tumefaciens*, CaMV and FMV), the regulatory sequences comprise a small part of their total genome, and in themselves have no pathogenic properties.

7.3 Relevant agricultural practices

243. It is anticipated that the agronomic practices for the cultivation of the GM canola will not differ from industry best practices used in Australia. The GM canola plants would therefore receive applications of water, fertilisers, herbicides, insecticides and other agronomic management practices similar to other commercially grown canola plants. Herbicides will be applied according to label directions. Standard cultivation practices for canola are discussed in more detail in *The Biology of* Brassica napus *L. (canola)* (OGTR 2011).

244. Growers of InVigor®, Roundup Ready® or InVigor® x Roundup Ready® canola are required to follow the relevant Crop Management Plan, as discussed in Section 5.5. These plans include management strategies that aim to control canola volunteers, minimise gene flow, and prevent the development of herbicide tolerant weeds.

245. In Australia, spring varieties of canola are 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. Canola is harvested either by windrowing (swathing) or by direct harvesting. Windrowing involves cutting the crop and placing it in rows to dry. The windrow lies in horizontal bundles, supported by the cut stems 10 - 20 cm off the ground, and remains in the paddock for 8 to 19 days prior to harvest. When most of the seed has matured and the moisture content is 9% or less, the windrow is picked up by the harvester (DPI Vic 2009; GRDC 2010).

Section 8 Australian and international approvals

8.1 Australian approvals of InVigor® x Roundup Ready® canola and related GMOs

8.1.1 Previous releases approved by the Gene Technology Regulator or authorised by the Genetic Manipulation Advisory Committee

246. InVigor® x Roundup Ready® canola has been approved by the Regulator for limited and controlled release (field trials) under licences DIR 069/2006 and DIR 104.

247. Field trials of InVigor® canola began in Australia in 1996. The first field trials were overseen by the Genetic Manipulation Advisory Committee (GMAC) as Planned Releases (PR) PR-62, PR-63 and their respective extensions. Under the current regulatory system, trials were subsequently approved by the Regulator under licence DIR 010/2001. Commercial release of InVigor® Hybrid canola was approved by the Regulator in 2003 under licence DIR 021/2002. As yet, InVigor® Hybrid canola has not been commercially grown in Australia.

248. Field trials of Roundup Ready® canola began in Australia in 1997. The trials were overseen by GMAC as PR-77 and associated extensions and were approved by the Regulator under licence DIR 011/2001. Commercial release of Roundup Ready® canola was approved by the Regulator in 2003 under licence DIR 020/2002. Commercial production began in New South Wales and Victoria in 2008 and in Western Australia in 2010.

249. In total, the Regulator has issued seven licences for the limited and controlled release of various GM canola lines (see www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1). In addition, there have been field trials of GM canola lines with various traits under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC) (see RARMP for DIR 103 for further detail).

250. There have been no credible reports of adverse effects on human health or the environment resulting from any of these releases.

8.1.2 Approvals by other Australian government agencies

251. Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. 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, including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and Australian Quarantine Inspection Service (AQIS)⁹.

252. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has approved the use of food derived from Roundup Ready® canola and the GM canola lines approved under licence DIR 021/2002. These approvals are listed in the Schedule to Standard 1.5.2 of the Australia New Zealand Food Standards Code under Items 1.1 (RoundupReady®) and 1.2 (InVigor®). These approvals were gazetted in December 2000

⁹ More information on Australia's integrated regulatory framework for gene technology is contained in <u>the *Risk Analysis Framework*</u> available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030.

and May 2002, respectively. FSANZ has determined that food derived from these GM lines of canola is as safe for human consumption as food derived from conventional canola (non-GM) varieties (ANZFA 2000; ANZFA 2001b). These approvals also cover InVigor® x Roundup Ready® canola.

253. APVMA has regulatory responsibility for the supply of agricultural chemicals, including herbicides and insecticidal products, in Australia. Bayer has been granted registration of glufosinate ammonium containing products for use on InVigor® canola (Liberty®) and Liberty Link® cotton (Liberty® 150 and Liberty® 200). Glufosinate ammonium containing products are not registered for use in any other broad-acre cropping in Australia. Glufosinate ammonium is also the active ingredient in the products Basta® and Finale® registered for weed control in horticultural and viticultural crops and in non-crop agricultural areas, commercial and industrial areas and rights-of-way.

254. Glyphosate is a broad-spectrum herbicide and is the active constituent of a range of proprietary herbicides registered by the APVMA, including Roundup Ready® herbicides for use on Roundup Ready® canola and Roundup Ready® cotton. Glyphosate has been registered for use in non-selective (general) weed control in broad-acre agriculture, horticulture and non-cropped areas including industrial areas and roadsides and is a widely used chemical in all these situations.

255. The APVMA have advised that amendments to the labels of glufosinate ammonium and glyphosate herbicide would be required for their use on commercial scale plantings of InVigor® x Roundup Ready® canola.

256. In addition, dealings authorised by the Regulator may be subject to the operation of State and Territory legislation declaring areas to be GM, GM free, or both, for marketing purposes. The Act allows for areas to be designated under State and Territory law for the purpose of preserving the identity of non-GM or GM crops for marketing purposes. Following the Regulator's approval in 2003 of GM InVigor® canola and GM Roundup Ready® canola on human health and environmental safety grounds, all jurisdictions except Queensland and the Northern Territory enacted legislation to delay the commercial release of GM crops, including GM canola, until marketability, agricultural trade and segregation issues were better understood. Subsequently, GM canola approved by the Regulator has been allowed to be commercially cultivated in New South Wales, Victoria and Western Australia.

8.2 International approvals

257. InVigor® x Roundup Ready® canola proposed for release is approved for commercial release in the USA and Canada, but has not yet been grown commercially.

258. The parental GM canola lines MS8, RF3, MS8 x RF3 and GT73 have been approved for commercial release in Canada, the USA and Japan. GM InVigor® canola and GM Roundup Ready® canola have been grown commercially in North America since 1995 and 1996, respectively.

Chapter 2 Risk assessment

Section 1 Introduction

259. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 2). Risks are identified within the context established for the risk assessment (see Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.



Figure 2 The risk assessment process

260. 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 (risk scenarios).

261. Each risk scenario is evaluated to identify those risks that warrant detailed characterisation. A risk is only identified for further assessment when a risk scenario is considered to have some reasonable chance of causing harm over either the short or long term. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

262. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2009c). In conjunction with these techniques, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

263. Identified 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). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments.

Section 2 Risk Identification

264. The following factors are taken into account when postulating relevant risk scenarios:

- the proposed dealings, which may be to conduct experiments, develop, produce, breed, propagate, grow, import, transport or dispose of the GMOs, use the GMOs in the course of manufacture of a thing that is not the GMO, and the possession, supply and use of the GMOs in the course of any of these dealings.
- the proposed limits, if any
- the proposed controls, if any
- characteristics of the parent organism(s)
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs
- 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.

265. Five risk scenarios were identified and evaluated in the context of the large scale of the release proposed by the applicant and in the absence of proposed limits and controls. These are summarised in Table 13, where circumstances that share a number of common features are grouped together in broader risk categories. None of the risk scenarios were identified as a risk that could be greater than negligible. Therefore, they did not warrant further detailed assessment. More detail of the evaluation of these scenarios is provided later in this Section.

266. Some of the hybrid GM canolas proposed for release contain the antibiotic resistance marker gene *nptII*. The *nptII* gene and its product has already been considered in detail in previous RARMPs, including for DIR 021/2002 and also for the commercial release of cotton (see DIR 12/2002, DIR 022/2002, DIR 023/2002, and DIR 059/2005), and by other regulators (for example EFSA 2007). Since *nptII* has been found to pose no risks to either people or the environment, its potential effects will not be further assessed for this application.

267. As the GMOs are derived by conventional crossing, the risks from unintended changes to the biochemistry (including innate toxic or allergenic compounds), physiology or ecology of the GMOs are not expected to be greater than the parental GMOs, which were assessed as negligible (see DIR 020/2002 and DIR 021/2002). There is no evidence or reasonable expectation that interactive or additive effects are likely to occur in the hybrid canolas proposed for release as all of the proteins encoded by the introduced genes operate through independent biochemical pathways. Therefore, unintended changes will not be further assessed for this application.

268. All of the introduced regulatory sequences are derived from common plants, bacteria and viruses. Similar regulatory elements are naturally present in canola, and the introduced elements operate in same way as endogenous ones. Although the transfer of introduced regulatory sequences into new genetic contexts, either in other plants or other organisms,

could result in unpredictable effects, the likelihood and impact of transfer of the introduced regulatory elements will not be different to those from endogenous regulatory elements. Hence these potential effects will not be further assessed for this application.

Table 13	Summary of risk scenarios from dealings with canola genetically modified for
herbicide	tolerance and a hybrid breeding system (InVigor® x Roundup Ready® canola)

	Risk scenario		Identified	
Risk category	Pathway that may give rise to harm	Potential harm	risk?	Reason
Section 2.1 Production of a toxic or allergenic substance	1. Exposure to GM plant material containing the proteins encoded by the introduced genes.	Allergic reactions in people or toxicity in people and other organisms	No	 The GM canola proposed for release is the product of conventional breeding between GM canola lines already assessed and approved by the Regulator for commercial release. The Regulator previously concluded that the parental GM canola lines were as safe as conventional canola. The hybrid canola proposed for release is not expected to be any more toxic or allergenic that the parental lines. Products derived from InVigor® x Roundup Ready® are approved by FSANZ for use in human food.
Section 2.2 The potential for spread and persistence of the GM canola plants in the environment	2. Expression of the introduced genes for herbicide tolerance and a hybrid breeding system increasing the invasiveness of the GM canola.	Weediness; allergic reactions in people or toxicity in people and other organisms	No	 The genetic modifications are not expected to alter the response of GM canola to biotic and abiotic stresses that naturally limit the geographical distribution of the species. The genetic modifications are expected to increase the fitness of GM canola plants in managed environments, but only when the corresponding herbicide(s) is applied. Canola plants with tolerance to both glufosinate ammonium and glyphosate can still be controlled by other herbicides or mechanical means.

	Risk scenario		Identified	
Risk category	Pathway that may give rise to harm	Potential harm	risk?	Reason
Section 2.3 Vertical transfer of genes to sexually compatible plants	3. Expression of the introduced genes in other canola plants	Weediness; allergic reactions in people or toxicity in people and other organisms	No	 Risk scenarios 1 and 2 associated with expression of the introduced genes did not constitute identified risks for people or the environment. The resulting GMO will be similar to GM InVigor® x Roundup Ready®, so no new adverse outcomes would occur. The genetic modifications are not expected to alter the response of GM canola to biotic and abiotic stresses that naturally limit the geographical distribution of the species. The genetic modifications are expected to increase the fitness of GM canola plants in managed environments, but only when the corresponding herbicide(s) is applied. Canola plants with tolerance to both glufosinate ammonium and glyphosate can still be controlled by other herbicides or mechanical means.
	4. Expression of the introduced genes in other sexually compatible plants	Weediness; allergic reactions in people or toxicity in people and other organisms	No	 Risk scenarios 1 and 2 associated with expression of the introduced genes did not constitute identified risks for people or the environment. Only low levels of gene transfer to plants in close proximity are likely to occur. Plants with tolerance to glufosinate ammonium and glyphosate can still be controlled by other herbicides or mechanical means.
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	5. Presence of the introduced genetic material in other organisms as a result of horizontal gene transfer	Weediness; allergic reactions in people or toxicity in people and other organisms	No	 The introduced genes and regulatory sequences are already present in the environment and are available for transfer via demonstrated natural mechanisms. Risk scenarios 1 – 4 associated with expression of the introduced genes did not constitute identified risks for people or the environment.

2.1 Production of a toxic or allergenic substance

269. 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).

270. 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).

271. A range of organisms may be exposed directly or indirectly to the proteins (and end products) encoded by the introduced genes for herbicide tolerance and a hybrid breeding system. Workers cultivating the GM canola would be exposed to all plant parts. FSANZ has approved the use of food derived from GM InVigor® canola and GM Roundup Ready® canola for human consumption (ANZFA 2000; ANZFA 2001b). These approvals also cover GM InVigor® x Roundup Ready® canola and therefore this is a potential source of exposure to people. Organisms may be exposed directly to the proteins through biotic interactions with GM canola plants (vertebrates, invertebrates, symbiotic microorganisms and/or pathogenic fungi), or through contact with root exudates or dead plant material (soil biota) or indirectly through the food chain.

Risk scenario 1. Exposure to GM plant material containing the proteins encoded by the introduced genes

272. Expression of the introduced genes for herbicide tolerance and a hybrid breeding system could potentially result in the production of novel toxic or allergenic compounds in the GM canola plants, or alter the expression of endogenous canola proteins. If humans or other organisms were exposed to the resulting compounds through ingestion, contact or inhalation of the GM plant materials, this may give rise to detrimental biochemical or physiological effects on the health of these people or other organisms.

273. The genes for herbicide tolerance and a hybrid breeding system introduced into the parental GM canola lines were all isolated from common soil bacteria, which are widespread and prevalent in the environment (see Chapter 1, Section 5.1.3). In addition, all of the parental GM canola lines are approved for commercial release (under DIR 020/2002 and DIR 021/2002), including for use in stockfeed. The CP4 EPSPS and PAT proteins are also present in GM cotton lines that have been approved for use in stockfeed since 2000 and 2006, respectively (see DIR 023/2002 and DIR 062/2005). Therefore, people and other organisms are already exposed to all of the proteins encoded by the introduced genes.

274. The toxicity of the parental GM canola lines was assessed in the RARMPs prepared for DIR 020/2002 and DIR 021/2002. This information was summarised and updated in Chapter 1, Section 5.4. The GM hybrid canolas proposed for release are not expected to be any more toxic or allergenic than the parental lines, as the same genes will be expressed under the control of the same regulatory elements. The novel proteins and their end products will be the same in the progeny of conventional breeding between the GM canola lines approved under licence DIR 021/2002 and Roundup Ready® canola as in the parental lines. Protein expression levels in InVigor® x Roundup Ready® canola are either similar to or lower than the low levels observed in the parental lines (see Chapter 1, Section 6.2.2). The proteins encoded by the introduced genes are well characterised and are not known to be toxic or allergenic (see Chapter 1, Section 5.1.3).

275. There is no evidence or reasonable expectation that interactive or additive effects are likely to occur in the hybrid canolas proposed for release or that they would result in new or increased risks relating to toxicity or allergenicity. The GOXv247 and CP4 EPSPS proteins present in Roundup Ready® canola operate through independent biochemical pathways unrelated to those of the BARNASE, BARSTAR or PAT proteins present in InVigor® canola. The *goxv247* and *cp4 epsps* genes are not expected to interact with any of the genes present in InVigor® canola, their proteins or their metabolic pathways.

276. Analysis of the compositional data for canola seed from InVigor® x Roundup Ready® canola indicates that there are no meaningful differences in the levels of compounds, including natural toxicants, when compared to non-GM canola from the same hybrid background and to other commercial canola varieties. Overall, the agronomic characteristics

of InVigor® x Roundup Ready® canola are comparable to their commercial MS8 x RF3 counterparts. These results indirectly support the lack of any interactive effects in hybrids resulting from conventional breeding between InVigor® canola lines and Roundup Ready® canola.

277. FSANZ has approved the use of food derived from GM InVigor® canola and GM Roundup Ready® canola for human consumption (ANZFA 2000; ANZFA 2001b). These approvals also cover GM InVigor® x Roundup Ready® canola.

278. *Conclusion*: The potential for allergic reactions in people, or toxicity in people and other organisms as a result of exposure to GM plant materials containing the proteins encoded by the introduced genes is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

2.2 The potential for spread and persistence of the GM canola in the environment

279. This section addresses the question of whether or not the proposed dealings with the GMOs may lead to harm to human health and safety or the environment as a result of an increased potential for spread and/or persistence due to the genetic modification.

280. All plants have the potential to lead to harm in certain environments. Harms that may arise from a certain plant species in a particular environment include:

- adverse effects on the health of people and/or animals
- reduction in the establishment, yield and/or quality of desired plants
- restriction in the physical movement of people, animals, vehicles, machinery and/or water
- adverse effects on environmental health, such as adverse changes to strata levels, nutrient levels, fire regime, soil salinity, soil stability, or by providing food and/or shelter to pests, pathogens and/or diseases.

281. For the purpose of this document, plant species causing significant levels of one or more of these harms are called 'weeds'. A plant species may be weedy in one or more land uses, such as dryland cropping or nature conservation.

282. Characteristics that influence the spread (dispersal of the plant or its genetic material) and persistence (establishment, survival and reproduction) of a plant species impact on the degree of its invasiveness. These characteristics include the ability to establish in competition with other plants, to tolerate standard weed management practices, to reproduce quickly, prolifically and asexually as well as sexually, and to be dispersed over long distances by natural and/or human means. The degree of invasiveness of a plant species in a particular environment gives an indication of the likelihood of its weediness in that environment. In addition to local experience, a history of weediness overseas can be used as an indicator for weediness in Australia.

283. Baseline information on the weediness of canola, including factors limiting the spread and persistence of non-GM canola plants, is given in *The Biology of* Brassica napus *L. (canola)* (OGTR 2011). In summary, canola is considered a major weed (naturalised and known to be a major problem at 4 or more locations within a State or Territory) in agricultural ecosystems in Australia (Groves et al. 2003). Surveys have shown that canola occurs as a volunteer weed in up to 10% of cereal crops in southern Australia (Lemerle et al. 1996). However, canola is not considered a significant weed nor invasive of natural undisturbed habitats in Australia (Dignam 2001; Groves et al. 2003). The weediness of the parental GM

canola lines was assessed in the RARMPs prepared for DIR 020/2002 and DIR 021/2002. This information was summarised and updated in Chapter 1, Section 5.5.

284. Scenarios relating to altered spread and persistence of the GM canola, compared to non-GM canola, include expression of the introduced genes for herbicide tolerance and a hybrid breeding system increasing the invasiveness of the GM canola.

Risk scenario 2. Expression of the introduced genes for herbicide tolerance and a hybrid breeding system increasing the invasiveness of the GM canola

285. If the GM canola plants were to establish or persist in the environment, the exposure of humans and other organisms to the GM plant material could be increased. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of contact with GM plant materials was discussed in Risk scenario 1 and was not considered an identified risk.

286. If the expression of the introduced genes for herbicide tolerance and a hybrid breeding system were to provide the GM canola plants with a significant selective advantage over commercially released canola plants and if they were able to establish and persist in non-cropped disturbed habitats and undisturbed natural habitats, this may give rise to lower abundance of desirable species, reduced species richness, or undesirable changes in species composition. Similarly, the GM canola plants could adversely affect cultivated areas if they exhibited a greater ability to establish and persist than commercially released canola.

287. As canola does not reproduce vegetatively under natural conditions, the most likely method of dispersal is via seed. Pod shattering can disperse seeds over short distances. It is also possible that GM canola plant material from windrows, including seed, could be blown beyond field boundaries. Dispersal distance would depend on the wind strength, the amount of trash on the ground and the moisture content of the material.

288. Dispersal of viable seed further from cultivated areas could occur in a variety of ways including endozoochory (dispersal through ingestion by animals), the activity of animals such as rodents and herbivores, through extremes of weather such as flooding or high winds, or via spillage during transport. If InVigor® x Roundup Ready® canola were commercialised, its distribution in unmanaged areas adjacent to fields and along transportation corridors would be expected to be comparable to that of non-GM volunteers.

289. The geographic range of non-GM canola in Australia is limited by a number of biotic and abiotic factors, including disease pressure, water and nutrient availability (OGTR 2011). As discussed in Chapter 1, Section 6.2.3, the agronomic characteristics of MS8 x RF3 x GT73 hybrids were comparable to their commercial MS8 x RF3 counterparts, apart from a small delay to maturity. Minimum and maximum values reported for MS8 x RF3 x GT73 plants were well within the range of values reported for the commercial hybrids. The production of the MS8 x RF3 x GT73 hybrids is not expected to alter the tolerance of plants to biotic or abiotic stresses that normally restrict geographic range and persistence of canola in natural habitats.

290. The weediness of the parental GM canola lines was assessed in the RARMPs prepared for DIR 020/2002 and DIR 021/2002. This information was summarised and updated in Chapter 1, Section 5.5. In summary, the introduced genes do not increase the potential weediness of the parental GM canola lines or provide these plants with an ecological advantage over non-GM canola, except in the presence of glyphosate (for Roundup Ready® canola) or glufosinate ammonium (for InVigor® canola). The GM hybrid canolas proposed for release are not expected to have any additional weediness traits, as the same genes will be

expressed under the control of the same regulatory elements. Canola tolerant to glufosinate ammonium and glyphosate are no more competitive than the parent single herbicide tolerant plants (Simard et al. 2005).

291. All GM canolas proposed for release will contain two herbicide tolerance traits. Expression of these traits will confer a selective advantage over non-GM counterparts in environments in which the corresponding herbicide is applied, such as agricultural settings and along roadsides. As the mode of action of each gene is herbicide-specific, cross-tolerance to other herbicides is not expected in the GM lines. Glufosinate ammonium is not widely used in broad-acre cropping or management of disturbed areas, so control options for the InVigor® x Roundup Ready® canola in these areas will be similar to those currently available to control Roundup Ready® canola. The management of InVigor® x Roundup Ready® canola on roadsides and other disturbed habitats could be achieved by the variety of management strategies available, including a range of alternative herbicides, tank mixing, and non-chemical management methods such as mowing, slashing, cultivation, burning and grazing.

292. 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, which is based on the resistance risk of each group of herbicides (CropLife Australia 2011). Glyphosate is a Group M herbicide and glufosinate ammonium is in Group N. Herbicides from different mode of action groups or products with multiple mode of action groups could be used to control InVigor® x Roundup Ready® volunteers. Specifically, herbicides from Groups B, C, F, G, H, I, L, O and Q are registered for use on canola in various crop and non-crop situations by the APVMA. In addition, several herbicides with multiple mode of action groups (eg Groups B + I, C + F, C + H, C + I, F + I, H + I, Q + L and K + B) are also registered for use on canola volunteers. Further details of registered herbicide products are available on the <u>APVMA website</u>.

293. The use of alternative herbicides for the control of InVigor® x Roundup Ready® canola volunteers may raise concerns that these herbicides could be more toxic or more persistent than glyphosate or glufosinate-ammonium. However, the APVMA registers herbicides on the basis that, when used as specified on the approved label, they will not compromise the health of users or the environment. The APVMA also has a program for reporting any adverse effects associated with agricultural chemical use and a program to review already registered agricultural chemicals.

294. When the weed risk potential of the GMOs is assessed based on the National Post-Border Weed Risk Management Protocol, they are considered to have no higher rating in terms of invasiveness or negative impacts than non-GM canola (see Chapter 1, Section 4.2) or the GM parental lines (see Chapter 1, Section 5.5).

295. *Conclusion*: The potential for improved survival of the GM canola through the expression of the introduced genes leading to increased spread and persistence in the environment is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

2.3 Vertical transfer of genes to sexually compatible plants

296. Vertical gene flow is the transfer of genes from an individual organism to its progeny by conventional heredity mechanisms, both asexual and sexual. In flowering plants, pollen dispersal is the main mode of gene flow (reviewed in Waines & Hegde 2003). For GM plants, vertical gene flow could therefore occur via successful cross pollination between the plant and neighbouring plants, related weeds or native plants (Glover 2002).

297. It should be noted that vertical gene flow *per se* is not considered an adverse outcome, but may be a link in a chain of events that may lead to an adverse outcome. For an increased potential for adverse effects to arise as a result of gene flow of the introduced genetic elements from the GM canola to sexually compatible plants, both of the following steps must occur:

- transfer of the introduced genetic elements to sexually compatible plants
- increased potential for adverse effects, such as toxicity or spread and persistence of the recipient plants, due to expression of the introduced gene.

298. Baseline information on vertical gene transfer associated with non-GM canola plants can be found in *The Biology of* Brassica napus *L. (canola)* (OGTR 2011) and in the RARMP prepared for DIR 105. In summary, canola is predominantly self-pollinating with average inter-plant outcrossing rates of 30%. Outcrossing frequencies are highest in the first 10 m of the recipient fields, and rates decline with distance (Husken & Dietz-Pfeilstetter 2007).

299. InVigor® x Roundup Ready® canola was generated by conventional crossing of three genetic modification events and, as expected, the events have been inserted into different regions of the plant genome and therefore segregate independently of one another. This means, after any initial out-crossing of InVigor® x Roundup Ready® canola, any subsequent generations may contain the same genes as either InVigor® or Roundup Ready® canola. Transfer of these single events into sexually compatible species was considered prior to approval of licences for DIR020/2002 and 021/2002 and the risks were considered negligible.

Risk scenario 3. Expression of the introduced genes in other canola plants

300. Transfer and expression of the introduced genes for herbicide tolerance and a hybrid breeding system to other canola plants could increase the weediness potential, or alter the potential allergenicity and/or toxicity of the resulting plants.

301. As discussed in Risk scenario 1, allergenicity to people and toxicity to people and other organisms are not expected to be changed in the hybrid GM canola plants by the combination of introduced genes. This will also be the case if the introduced genes are expressed in other canola plants.

302. As discussed in Risk scenario 2, the genes introduced into the hybrid GM canola plants are not expected to alter the tolerance of plants to biotic or abiotic stresses that normally restrict geographic range and persistence of canola in natural habitats. Similarly, they would not be expected to alter the geographic range or persistence of other canola plants if the introduced traits were transferred to their progeny.

303. However, the two herbicide tolerance genes present in the GM canola plants would confer a selective advantage in areas where the corresponding herbicides are applied. This would also be true if the traits were conferred to other canola plants in the environment.

304. In the broad-acre field situation, cross pollination between the hybrid GM canola proposed for release and other canola would be most likely to occur when canola crops are grown in adjacent paddocks and flower synchronously. Cross pollination may also occur where volunteer plants emerge after canola crops are harvested and develop to flowering stage, or where feral canola populations, resulting from seed being dispersed off-farm, establish along roadsides adjacent to cropping land where canola is planted.

Gene transfer to GM canola

305. Gene transfer could occur to other GM canola approved for either commercial or limited and controlled release. These include:

- Limited and controlled releases under DIRs 032/2002, 069/2006, 103, 104 and 105, or future limited and controlled release licences
- Commercial releases under DIR 020/2001 (Roundup Ready® canola) and DIR 021/2002 (InVigor® canola)
- Other GM canolas which may be approved for commercial release in the future.

306. Licence conditions for limited and controlled GM canola releases include measures to restrict gene flow. Additionally, controls placed on GM canola released under limited and controlled conditions include not using the GMOs in food or feed and destroying any GMOs and volunteer plants in the areas of the release in accordance with the licence. Therefore, the potential for any adverse outcome from gene transfer to these limited and controlled releases of GM canola as a result of the proposed dealings is considered negligible.

307. The only GM canolas currently approved for commercial release are the parent lines of the GMOs proposed for release. Outcrossing of InVigor® x Roundup Ready® to these commercially approved GM canola plants would result in plants highly similar to the GMOs proposed for release. Therefore, any adverse outcomes expected for those progeny would be comparable to InVigor® x Roundup Ready® canola.

308. Gene transfer could also occur to other GM canolas approved for commercial release in the future, which would lead to stacking of the genetic modifications. If any other canola was proposed for commercial release in the future, a risk assessment would be conducted taking into account potential stacking with already approved GM varieties. This would include consideration of potential interactions between different GM traits.

Gene transfer to non-GM, non-herbicide tolerant canola

309. Gene transfer to non-GM, non-herbicide tolerant canola plants would result in plants highly similar to the GMOs proposed for release or to their GM parents approved under DIR 020/2002 and 021/2002. Therefore, any adverse outcomes expected for those progeny would be comparable to InVigor® x Roundup Ready® canola or their parental GM canola lines. The control of glyphosate tolerant and glufosinate ammonium tolerant canola volunteers that occur as a result of gene transfer from InVigor® x Roundup Ready® canola crops represents an agricultural production issue with potential economic impact in terms of alternative weed management choices. There are a range of alternative herbicides assessed and approved by the APVMA which can be used to control GM canola volunteers (as described in Risk Scenario 2) in addition to mechanical means.

Gene transfer to non-GM herbicide tolerant canola

310. There are two conventionally bred herbicide-tolerant canola varieties currently being widely grown in Australia – triazine tolerant (TT) and imidazolinone-tolerant (Clearfield®). Where canola varieties that are tolerant to different herbicides are in close proximity, the production of multiple-herbicide resistant volunteers has been noted (Hall et al. 2000; Beckie et al. 2003; Knispel et al. 2008; Schafer et al. 2011). Gene transfer from InVigor® x Roundup Ready® canola to non-GM herbicide tolerant canola could result in the stacking of genes for tolerance to up to four different herbicide groups. Although InVigor® canola has not been commercially grown in Australia, this stacking of four herbicide tolerance traits has been a possibility since the approval of InVigor® canola and Roundup Ready® canola in 2003. Stacking was considered in the RARMPs for DIR 020/2002 and 021/2002 and was assessed to pose negligible risks. However, if InVigor® x Roundup Ready® canola were commercialised, development of canola plants with all four herbicide tolerance traits would be more likely, as it would require only two rather than three separate hybridisation events.

311. Apart from being tolerant to additional herbicides, such stacked GM canola is not expected to differ from the parental GM and non-GM varieties. There is no evidence or reasonable expectation that the non-GM herbicide tolerance traits would interact with the introduced genes from the GM canola proposed for release leading to changes in toxicity, allergenicity or weediness.

312. Multiple-herbicide tolerant individuals are as susceptible to alternative herbicides as are single-herbicide tolerant canola plants or their non-GM counterparts (Senior et al. 2002; Beckie et al. 2004; Dietz-Pfeilstetter & Zwerger 2009). In laboratory studies, multiple-herbicide tolerant canola plants were no more competitive than single-herbicide tolerant controls (Simard et al. 2005). Therefore, if multiple-herbicide tolerant canola plants were to occur, they are unlikely to be more invasive or persistent than non-herbicide tolerant or single-herbicide tolerant canola plants and could be controlled by other herbicides or other (non-chemical) agricultural practices. Triazine herbicides are in mode of action Group C, and imidazoline herbicides are in Group B. As discussed in Risk Scenario 2, there are a range of other herbicide products available with alternative or multiple modes of action.

313. Management of the impacts of gene transfer from InVigor® x Roundup Ready® canola to other canola can be achieved by the application of the already established principles and practices for minimising the development of herbicide resistance in any agricultural weeds: attention to the control of volunteers; informed selection and rotation of herbicides and crops; maintenance of hygiene in seeding, harvesting and transport operations; and implementation of good agronomic practices (Rieger et al. 2001; Downey 1999; Salisbury 2002c). These measures are incorporated in the Crop Management Plans that growers of InVigor® canola or Roundup Ready® canola are obliged to follow, and which will be implemented for InVigor® x Roundup Ready® canola.

314. While the control of canola with multiple herbicide tolerance traits may represent an agricultural production issue with potential economic impacts in terms of alternative weed management choices, there remains a range of approved herbicides and non-chemical methods of control.

315. *Conclusion*: The potential for allergenicity in people, or toxicity in people and other organisms, or increased weediness due to expression of the introduced genes in other canola plants as a result of gene transfer is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

Risk scenario 4. Expression of the introduced genes in other sexually compatible plants

316. Transfer and expression of the introduced genes for herbicide tolerance and a hybrid breeding system in other sexually compatible plants could increase the weediness potential, or alter the potential allergenicity and/or toxicity of the resulting plants. As discussed in Risk scenario 1, the introduced genes do not encode proteins that are considered toxic or allergenic. Therefore, even if the introduced genes were to be transferred to, and expressed in, sexually compatible species, the recipient species would likely be no more toxic or allergenic than their unmodified precursors.

317. Under natural conditions, canola can cross with cultivated *Brassica* species (*B. napus*, *B. juncea, B. rapa* and *B. oleracea*) and, at very low frequencies, with three weed species important in Australia (*R. raphanistrum*, *H. incana* and *S. arvensis*) (Salisbury 2002b).

318. The risks associated with transfer of the introduced genes from the parental GM canola lines was previously assessed as very low to negligible, as summarised in Chapter 1, Section 5.6.2. The GM canolas proposed for release were produced by conventional breeding,

and the potential for gene flow from them to compatible species, and the fitness of the resulting hybrids, is expected to be as low as for the parental GM canola lines.

319. The only difference in the consequence of gene flow from the GM canola proposed for release and the parental GM canola lines is the potential for the transfer of genes conferring tolerance to two herbicides in a single cross pollination event. This may confer a selective advantage in cultivated areas and non-cropped disturbed habitats where the corresponding herbicides are applied. However, these plants could be controlled using the range of alternative herbicides and non-chemical management techniques currently used in integrated weed management to control brassicaceous weeds and canola volunteers.

320. *Conclusion:* The potential for allergenicity in people, toxicity in people and other organisms or increased weediness due to the expression of the introduced genes in other sexually compatible plant species as a result of gene transfer is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms

321. Horizontal gene transfer (HGT) is the stable transfer of genetic material from one organism to another without reproduction (Keese 2008). Data are accumulating to show that HGT is more widespread than previously believed and has been a significant force in the evolution of eukaryotic genomes (Bock 2010). In general, HGT between multicellular eukaryotes appears to be rare, occurring only on an evolutionary timescale, but has occurred between plants as well as between plants and less complex organisms (Bock 2010). All genes within an organism, including those introduced by gene technology, are capable of being transferred to another organism by HGT. HGT itself is not considered an adverse effect, but could be part of a scenario potentially leading to harm. A gene transferred through HGT could confer a novel trait to the recipient organism, through expression of the gene itself or by altering the expression of endogenous genes. The novel trait may result in negative, neutral or positive effects.

322. Risks that might arise from horizontal gene transfer have been reviewed (Keese 2008) and considered in previous RARMPs (eg DIR 057/2004, DIR 085/2008 and DIR 091) which are available from the OGTR website http://www.ogtr.gov.au or by contacting the Office. From the current scientific evidence, HGT from GM plants to other organisms presents negligible risks to human health and safety or the environment. This is due to the rarity of such events, relative to those HGT events that occur in nature, and the limited chance of providing a selective advantage to the recipient organism that would promote the spread and persistence of the transferred material.

323. Baseline information on the presence of the introduced or similar genetic elements is provided in Chapter 1, Section 7.2.3. All of the introduced genetic elements are derived from naturally occurring organisms that are already present in the wider Australian environment.

Risk scenario 5. Presence of the introduced genetic material in other organisms as a result of horizontal gene transfer

324. Possible risks arising from HGT of the introduced genetic material to other organisms involves consideration of the potential recipient organisms and the nature of the introduced genetic material.

325. HGT could result in the presence of the introduced genes for herbicide tolerance, a ribonuclease and a corresponding ribonuclease inhibitor in bacteria, plants, animals or other eukaryotes. However, the introduced genes were isolated from common bacteria that are

widespread in the environment (See Chapter 1, Section 7.2.3). It is far more likely that horizontal gene transfer will occur from naturally occurring *B. amyloliquefaciens*, *S. hygroscopicus*, *S. viridochromogenes*, *O. anthropi* or *A. tumefaciens* bacteria than from the GM canola plants.

326. In addition, the introduced genes are present in the parental GM canola lines already approved for commercial release. The *bar*, *pat*, and *cp4 epsps* genes are also present in GM cotton approved for commercial release (for example see DIR 062/2005, DIR 066/2006 and DIR 091). Therefore, the introduced genes are already available for HGT from the source organisms or commercially approved GM plants.

327. The likelihood of gene transfer was recently found to be negligible in studies on HGT of the *cp4 epsps* gene from GM canola to microorganisms during digestion in ruminants and during *in vitro* incubations (Sharma et al. 2004; Alexander et al. 2006; Reuter et al. 2007; EFSA 2009c).

328. Furthermore, the introduced *bar*, *pat*, *cp4 epsps* and *goxv24* genes in the GM canola plants have been modified for plant codon usage, so in the unlikely event that gene transfer were to occur, only relatively low levels of gene expression in bacteria would be expected. The gene sequences expressed from the introduced genetic material are not expected to assist the process of HGT by facilitating gene movement across cell membranes or recombination with a host genome. Therefore, any rare occurrence of HGT of introduced genetic material to other organisms is not expected to persist and/or result in an adverse effect.

329. A key consideration in the risk assessment process should be the safety of the protein product resulting from the expression of the introduced gene rather than horizontal gene transfer *per se* (Thomson 2000). If the introduced genes, the encoded proteins or their end products are not associated with any risk then even in the unlikely event of HGT occurring, they should not pose any risk to humans, animals or the environment. Conclusions reached for Risk Scenarios 1 - 4 associated with the expression of the introduced genes did not represent an identified risk.

330. *Conclusion:* The potential for an adverse outcome as a result of horizontal gene transfer is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

Section 3 Risk estimate process

331. The risk assessment begins with postulation of credible pathways that might lead to harm to the health and safety of people or the environment during the proposed release of GMOs due to gene technology, and how it could happen, in comparison to the parent organism and within the context of the receiving environment.

332. Five risk scenarios were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms, or alter characteristics that may impact on the spread and persistence of the GM plants. The opportunity for gene flow to other organisms and its effects if it occurred were also assessed. These risk scenarios were considered over both the short and long term.

333. A **risk** is only identified when a risk scenario is considered to have some chance of causing harm. Risk scenarios that do not lead to harm, or could not reasonably occur, do not represent an identified risk and do not advance any further in the risk assessment process.

334. The characterisation of the five risk scenarios in relation to both the seriousness and likelihood of harm did not identify any risks that could be greater than negligible. Therefore, they did not warrant further detailed assessment. The principal reasons for this include:

- the GM canola has been produced by conventional breeding of GM canola lines that have previously been assessed and authorised for commercial release in Australia
- widespread presence of the same or similar proteins encoded by the introduced genes in the environment and lack of known toxicity or evidence of harm from them
- limited capacity of the GM canola to spread and persist in undisturbed natural habitats.

335. Therefore, any risks to the health and safety of people, or the environment, from the proposed release of the GM canola plants into the environment is considered to be **negligible**.

Section 4 Uncertainty

336. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communication. Both dimensions of risk (ie consequence and likelihood) are always uncertain to some degree.

337. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability¹⁰. For commercial/general releases, where there may not be limits and controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, uncertainty may be addressed through post release review (see Chapter 3, Section 4).

338. InVigor® x Roundup Ready® canola has been approved by the Regulator for limited and controlled release (field trials) under licences DIR 069/2006 and DIR 104. These licences also authorised the release of several other lines of GM canola as well as lines of GM Indian mustard (*Brassica juncea*). In the DIR 069/2006 and DIR 104 RARMPs, information was identified as requiring possible consideration if Bayer were to submit an application for a larger scale release of the GMOs. The information identified that is relevant to the GM canola lines can be categorised as follows:

- additional molecular and biochemical characterisation of the GM canola and Indian mustard lines (eg genotypic stability, and expression levels of the introduced genes)
- data on the potential toxicity of plant material from the GM canola and Indian mustard lines including levels of known endogenous toxins
- phenotypic characterisation of the GM canola and Indian mustard lines, particularly with respect to traits that may contribute to biotic or abiotic stress tolerance, weediness (eg germination and reproductive capacity) or persistence (eg seed dormancy)
- the level of pollen mediated gene flow between both canola and Indian mustard and related species in Australia

339. Bayer has submitted five reports characterising the InVigor® x Roundup Ready® canola proposed for commercialisation in Australia in relation to the first three points listed above. These have been discussed in Chapter 1.

340. No new data on the level of pollen mediated gene flow between canola and related plants in Australia was provided by the applicant. However, the uncertainty noted in the RARMP for DIR 104 was associated with the potential for pollen-mediated gene flow from

¹⁰ A more detailed discussion is contained in the <u>Regulator's *Risk Analysis Framework*</u> or via Free call 1800 181 030.

GM Indian mustard plants, rather than from the GM canola. Information on gene flow from canola is available in published literature, and the potential for gene flow between canola and sexually related plants is discussed in Risk Scenario 4.

341. For all commercial or long-term releases uncertainty exists in relation to changes in the context surrounding the release. The risk assessment has been prepared in the context of current agricultural practices, climate and weather patterns, and the conclusions are appropriate in this context, however, over time if these were to change then the appropriateness of these conclusions is less certain.

342. The level of uncertainty about InVigor® x Roundup Ready® canola is considered to be low given the now several years of growing the parental GM canola lines in Australia and overseas, eg in Canada and the USA.

Chapter 3 Risk management plan

Section 1 Background

343. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan evaluates and treats identified risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through proposed licence conditions.

344. 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.

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

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

Section 2 Risk treatment measures for identified risks

347. 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 GM canola. These risk scenarios were considered in the context of the large scale of the proposed release and the receiving environment and considering both the short and long term. The *Risk Analysis Framework* (OGTR 2009a), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation. Therefore, no conditions are imposed to treat these negligible risks.

Section 3 General risk management

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

- applicant suitability
- identification of the persons or classes of persons covered by the licence
- reporting structures
- a requirement that the applicant allows access to specified sites for purpose of monitoring or auditing.

3.1 Applicant suitability

349. 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.

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

351. The licence includes a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability or their capacity to meet the conditions of the licence.

352. Bayer must continue to have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

3.2 Testing methodology

353. Bayer is required to provide a method to the Regulator for the reliable detection of the presence of the GMOs and the introduced genetic materials in a recipient organism. This instrument is required within 30 days of the issue date of the licence.

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

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

3.4 Reporting requirements

355. 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.

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

357. 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

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

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

Section 4 Post release review

360. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator does not fix durations, but 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.

361. 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 be achieved through post release review (PRR) activities. The three components of PRR are:

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

362. 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

363. Any member of the public can report adverse experiences/effects resulting from an intentional release 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 4.3 below) as well as the risk assessment of future applications involving similar GMO(s).

4.2 Requirement to monitor specific indicators of harm

364. Additional specific information on the 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. Specific indicators of harm may also be identified at a later stage, eg through either of the other components of PRR.

365. 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. If specific indicators or harm were identified, licence holders would be required to monitor them as mandated by the licence.

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

367. The characterisation of the risk scenarios discussed in Chapter 2 did not identify any risks that could be greater than negligible and the level of uncertainty is considered to be low. Therefore, no specific indicators of harm have been identified in this RARMP for application DIR 108.

4.3 Review of the RARMP

368. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would be desktop-based and 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 needed managing, this could lead to changes to the licence conditions.

Section 5 Conclusions of the RARMP

369. The risk assessment concluded that this commercial release of GM canola poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

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

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Appendix A Summary of issues raised in submissions received from prescribed experts, agencies and authorities on any matters considered relevant to the preparation of a Risk Assessment and Risk Management Plan for DIR 108

The Regulator received a number of 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 where they are addressed in the consultation RARMP, are summarised below.

Summary of issues raised	Comment
 In preparing the RARMP the Regulator should consider: the potential for commercial scale growing of the GM canola to affect weediness. the potential for the GM canola to cross with existing non-GM herbicide tolerant canola and any possible associated risks to the environment. the potential for gene flow to related species and possible risk of weediness 	These issues were considered in Chapter 2 of the RARMP. The potential for expression of the introduced genes to lead to increased spread and persistence of the GM canola in the environment was assessed in the context of a commercial scale release in Risk Scenario 2 and was not identified as a risk that warranted further assessment. The potential for harm due to expression of the introduced genes in other related plants, including non-GM herbicide tolerant canola and weedy species, as a result of gene transfer was assessed in Risk Scenarios 3 and 4 and were not identified as risks that warranted further assessment.
Council has resolved to take neutral position on the use of GM crops.	Noted.
For marketing reasons, Council does not support the growing, storage and transport of GM crops within the Shire. Council noted that there is no current legislative power that enables any Council Officer to enforce the implementation or policing of this Policy.	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. Some areas may be designated under State or Territory law for the purpose of preserving the identity of GM or non-GM crops (or both) for marketing purposes. However, marketing issues are outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence.
The DNA of these plants has been altered and there have been no long term studies regarding the human health impacts of these products.	This issue was considered in Chapters 1 and 2 of the RARMP. Toxicity of the parental GM canola lines to humans is considered in Section 5.4.1. The conventionally bred GM canola proposed for release is not expected to be any more toxic than the parental lines as the same genes will be expressed. The proteins encoded by the introduced genes are well characterised and are not known to be toxic or allergenic. The potential for allergic reactions in people, or toxicity in people and other organisms, as a result of exposure to GM plant materials was assessed in Risk Scenario 1 and was not identified as a risk that warranted further assessment.
Some of the products containing GM canola include baby foods, potato chips and biscuits.	FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has approved the use of food derived from GM InVigor® canola and GM Roundup Ready® canola for human consumption. These approvals also cover GM InVigor® x Roundup Ready® canola.

Summary of issues raised	Comment
GM foods seem to be largely exempt from labelling requirements.	FSANZ is responsible for human food safety assessment and food labelling, including GM food.
The commercial release of GM canola into the environment may impact on non GM growers.	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. 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 are matters for States and Territories, and industry.
Based on the information presently known, Council continues to object to the growing of genetically modified (GM) crops in its area.	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 Regulator must not issue a licence unless satisfied that risks can be managed to protect human health and safety and the environment.
LGA deals with issues resulting from this product escaping bulk transport vehicles when carting along the roadway after harvesting. LGA has had reports of GM canola growing on roadsides and concerns about who is responsible for cleaning up/containing the re-growth. Given that 90% of our roadsides have a conservation rating of high to very high, anything that can be done to minimise the risk of grain regeneration on our roadsides is appreciated in order to: a) Reduce farmers worries about cross contamination & b) Council concerns about eradication of the product on its roadsides.	These issues were considered in Chapter 2 of the RARMP. The potential for expression of the introduced genes to lead to increased spread and persistence of the GM canola in the environment, including non- cropped disturbed habitats such as roadsides, was assessed in Risk Scenario 2 and was not identified as a risk that warranted further assessment. Further information on the control of volunteer GM canola on roadsides can be found in the <i>Control of roadside canola volunteers</i> fact sheet, available on the OGTR website.
GM canola is a concern for ratepayers who want the municipality to be a GM free zone.	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 Regulator must not issue a licence unless satisfied that risks can be managed to protect human health and safety and the environment. The RARMP for this release considered information provided by the applicant and the currently available scientific information, in the context of the large scale of the proposed release, and concluded that risks to human health and the environment are negligible. Some areas may be designated under State or Territory law for the purpose of preserving the identity of GM or non-GM crops (or both) for marketing purposes. However, marketing issues are outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence.
Due to variance of opinion, consensus by Councils on at least a regional basis must be attained. Continued haphazard and separate applications and/or approvals from/for various companies cannot be supported.	The Gene Technology Act 2000 allows a person to apply to the Gene Technology Regulator for a licence authorising specified dealings with one or more GMOs. The Regulator must consider an application and must issue the licence, or refuse to issue the licence, within a specified time period. Each application for a DIR licence is assessed on a 'case by case' basis.
In recent times there have been various media articles highlighting difficulties of treating roadside vegetation that is now immune to easy chemical treatment. It is suggested that this vegetation is GM.	This issue was considered in Chapter 2 of the RARMP. The potential for expression of the introduced genes to lead to increased spread and persistence of the GM canola in the environment, including non- cropped disturbed habitats such as roadsides, was assessed in Risk Scenario 2 and was not identified as a risk that warranted further assessment. Further information on the control of volunteer GM canola on roadsides can be found in the <i>Control of roadside canola volunteers</i> fact sheet, available on the OGTR website.

Summary of issues raised	Comment
LGA has adopted the precautionary principle as set out in the guiding principles of the Environment Protection Act. Hence it opposes trials of GM canola due to uncertainties and potential impacts of GMOs on health, environment and agriculture within our area. Concerned there is a lack of scientific certainty around the cumulative and compounding impacts of further modifying canola and/or products.	These issues were considered in Chapters 1 and 2 of the RARMP. The RARMP for this release considered information provided by the applicant and the currently available scientific information, in the context of the large scale of the proposed release, and concluded that risks to human health and the environment are negligible.
LGA is yet to be convinced that the release of GM products without significant direct benefits to public health should be permitted. Motto of "pure" provides a market advantage. Do not support growing, storage or transport of GM crops within this area in direct opposition to this marketing strategy.	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. Some areas may be designated under State or Territory law for the purpose of preserving the identity of GM or non-GM crops (or both) for marketing purposes. However, marketing issues are outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence.
Expect secure safeguards to be in place to prevent escape from trial areas and to ensure bees do not spread GM canola to other areas. As there is a concern that canola is considered a high risk crop for pollen mediated gene flow, further research is required to resolve that issue.	These issues were considered in Chapter 2 of the RARMP. Application DIR 108 is for the commercial release of GM canola. The potential for harm due to expression of the introduced genes in other canola plants as a result of gene transfer was assessed in Risk Scenario 3 and was not identified as a risk that warranted further assessment. This Risk Scenario included consideration of canola pollination by honeybees. Risk to human health and safety and the environment from the proposed release are assessed to be negligible. Therefore, the Regulator has not proposed any limits or controls to restrict the release.
LGA has significant variation in topography, soil, water and other physical attributes. Therefore locations of trial crops need to be disclosed to provide further meaningful response, particularly from people familiar with the area where the release could take place.	Application DIR 108 is for the commercial release of GM canola in all commercial canola growing areas of Australia.
LGA believes it is important that any GM application should receive a broad public notification/opportunity for comment so that informed choices can be made by more than just the regulators and those supporters for GM releases.	The Act requires extensive consultation on all DIR RARMPs with a wide range of experts, agencies and authorities, and with the public. The public invitation to comment must be published in the Commonwealth Gazette, in a national newspaper and on the OGTR website. The Regulator routinely exceeds these requirements by publishing the invitation to comment on the RARMP in regional newspapers as well as sending it to people and organisations that have registered on the OGTR mailing list. In finalising the RARMP and making a decision on whether or not to issue a licence, the Regulator must have regard to the submissions received.
Limited if any commercial growing of canola in the Shire and no expertise within council on this specialised subject. No comment is offered.	Noted.

Summary of issues raised	Comment
Council not involved in managing GM crops and has no expertise with regards to a response. Suggests consulting Western Australian Local Government Association (WALGA).	WALGA has been consulted.
Any impact on honey bees and honey production/labelling?	This issue was considered in Chapters 1 and 2 of the RARMP. The GM canola proposed for release is the product of conventional breeding between GM canola lines already assessed and approved by the Regulator for commercial release. The toxicity of the parental GM canola lines was discussed in Chapter 1. This discussion included consideration of toxicity to people, including via honey, and toxicity to honeybees. The potential for allergic reactions in people, or toxicity in people and other organisms, as a result of exposure to GM plant materials was assessed in Risk Scenario 1 and was not identified as a risk that warranted further assessment. The hybrid canola proposed for release is not expected to be any more toxic or allergenic than the parental lines. FSANZ is responsible for human food safety assessment and food labelling, including GM food. Products derived from InVigor® x Roundup Ready® are approved by FSANZ for use in human food.
Any impact on aquatic weeds?	This issue was considered in Chapter 1 of the RARMP. Apart from the herbicide tolerance traits, the GM canola has the same characteristics as non-GM canola and other already approved GM canola varieties. <i>Brassica napus</i> is not known to be able to hybridise with any aquatic weed species under natural conditions. Therefore, pollen mediated gene flow to aquatic species is highly unlikely.
Any impact of associated excessive use of Roundup then impacting on native vegetation and waterways?	Roundup Ready® canola, tolerant to glyphosate, is already approved for commercial release under DIR 020/2002. Issues relating to the use of herbicides are outside the scope of the Regulator's assessments. The APVMA has regulatory responsibility for the supply of agricultural chemicals, including herbicides, in Australia.
Any different impact if it strayed onto roadside reserves or Council land?	This issue was considered in Chapter 2 of the RARMP. The potential for expression of the introduced genes to lead to increased spread and persistence of the GM canola in the environment, including non- cropped disturbed habitats such as roadsides, was assessed in Risk Scenario 2 and was not identified as a risk that warranted further assessment. Further information on the control of volunteer GM canola on roadsides can be found in the <i>Control of roadside canola volunteers</i> fact sheet, available on the OGTR website.
Are there likely to be any changed impact on hayfever sufferers?	This issue was considered in Chapter 2 of the RARMP. The GM canola proposed for release is the product of conventional breeding between GM canola lines already assessed and approved by the Regulator for commercial release. The potential for allergic reactions in people, or toxicity in people and other organisms, as a result of exposure to GM plant materials was assessed in Risk Scenario 1 and was not identified as a risk that warranted further assessment. The hybrid canola proposed for release is not expected to be any more toxic or allergenic that the parental lines.

Summary of issues raised	Comment
Is this likely to impact on the amount of canola crops being grown at once? Amount of synchronized sediment run-off and even more mono-culture?	This issue was considered in Chapter 2 of the RARMP. Application DIR 108 is for the commercial release of GM canola in all commercial canola growing areas of Australia. The RARMP for this release concluded that risks to human health and the environment are negligible. Therefore, the Regulator has not proposed any limits or controls to restrict the release. If approved, this GM canola could be grown in NSW, Victoria, Queensland and WA. However, State government requirements imposed for marketing
	reasons would currently prevent this GM canola from being grown in SA and Tasmania. The introduced traits are not expected to alter the geographic range of where canola is currently grown.
Based on the precautionary principle, the approval of potentially high risk developments such as the commercial release of GM canola, should not occur until public and environmental safety can be guaranteed and the community has had an opportunity to be informed about and respond to risks they may be subjected to. Taking into account the uncertainty, more comprehensive safety assessment processes and extensive public consultation are required prior to the commercial release of this product.	These issues were considered in Chapters 1 and 2 of the RARMP. The RARMP for this release considered information provided by the applicant and currently available scientific information, and concluded that risks to human health and the environment are negligible. The Act requires extensive consultation on all DIR RARMPs with a wide range of experts, agencies and authorities, and with the public. The public invitation to comment must be published in the Commonwealth Gazette, in a national newspaper and on the OGTR website. The Regulator routinely exceeds these requirements by publishing the invitation to comment on the RARMP in regional newspapers as well as sending it to people and organisations that have registered on the OGTR mailing list. In finalising the RARMP and making a decision on whether or not to issue a licence, the Regulator must have regard to the submissions received.
Health risks that the release of GM canola may pose through consumption of food products or derivatives should be considered. The rights of the community to choose whether they consume GM foods based on an adequate labelling and measures to prevent contamination of non-GM crops. The wider community should be informed about the ingredients, and GM content, of the foods they consume. While noted that both InVigor and Roundup Ready have already been licensed for commercial release separately the two should be assessed separately as per the FSANZ' case-by-case basis, rather than being covered by existing approvals for each separate component. Given the difficulty of identifying all of the unintended expressions of genetic modification, it would be prudent for FSANZ to adopt amore rigorous safety assessment. Need for GM foods to be subjected to clinical trials in the same way pharmaceutical drugs are, including routine use of oral toxicity studies in animals. Some such studies have found evidence of adverse health effects.	These issues were considered in Chapters 1 and 2 of the RARMP. Toxicity of the parental GM canola lines to humans is considered in Chapter 1, Section 5.4.1. The conventionally bred GM canola proposed for release is not expected to be any more toxic or allergenic than the parental lines as the same genes will be expressed. The proteins encoded by the introduced genes are well characterised and are not known to be toxic or allergenic. The potential for allergic reactions in people, or toxicity in people and other organisms, as a result of exposure to GM plant materials was assessed in Risk Scenario 1 and was not identified as a risk that warranted further assessment. FSANZ is responsible for human food safety assessment and food labelling, including GM food. Products derived from InVigor® x Roundup Ready® are approved by FSANZ for use in human food. For more information on FSANZ GM food assessments, see FSANZ website (http://www.foodstandards.gov.au).

Summary of issues raised	Comment
 The impacts to flora and fauna of herbicides use on herbicide resistant GM crops and cross contamination to nearby crops should be considered. Herbicides have a range of undesirable environmental impacts, including: creation of 'super-weeds' that display glyphosate resistance acute toxic effects other health impacts from environmental exposure 	These issues were considered in Chapter 1 of the RARMP. Issues relating to the use of herbicides are outside the scope of the Regulator's assessments. The APVMA has regulatory responsibility for the supply of agricultural chemicals, including herbicides, in Australia. The development of herbicide resistant weeds was discussed in Chapter 1, Section 5.5.3. Herbicide resistance is managed by the APVMA under conditions of registration for the use of agricultural chemicals in Australia.
Impact on organic farms adjacent and/or in proximity to GM crops should be considered.	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. 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 are matters for States and Territories, and industry.
Do not have a formal position on this issue as there is no cropping area within the municipality.	Noted.
Notes that FSANZ has approved this canola to be used for human consumption. As this product will be used in food it should be clearly indicated on any labelling so that consumers can make informed choices	FSANZ is responsible for human food safety assessment and food labelling, including GM food. Products derived from InVigor® x Roundup Ready® are approved by FSANZ for use in human food.
Believes it is important that a licence protects the interests of Australian farmers and Australia's food security from any cross contamination. Trusts that such protection will be included in any permit granted for the release of GM canola into our environment.	This issue was considered in Chapter 3 of the RARMP. 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. 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 are matters for States and Territories, and industry. The risk assessment concluded that there are negligible risks to people and the environment from the proposed release of GM canola. Therefore, no specific licence conditions are imposed to treat these negligible risks.
Request that you err on the side of caution regarding commercial release of GM canola to protect human health and safety. Asks that the rights of farmers to farm without impediment that may arise from neighbouring GM crops be protected.	These issues were considered in Chapters 1 and 2 of the RARMP. The RARMP for this release considered information provided by the applicant and currently available scientific information, and concluded that risks to human health and the environment are negligible. 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. 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 are matters for States and Territories, and industry.
Supportive of the application.	NOTEO.

Summary of issues raised	Comment
The RARMP should focus significantly on new risks that may arise in this line of GM canola due to the synergistic effects of gene stacking. For example, the application identifies 2 risks, gene flow into other organisms such as weedy species and the emergence of canola volunteers tolerant to the herbicides. It is apparent that the effect of these events occurring in this stacked GM line is potentially different, as having two herbicide resistant traits present reduces the potential tools to manage resistance.	These issues were considered in Chapter 2 of the RARMP. The RARMP discusses the parental GM canola lines in Chapter 1, as part of the risk assessment context for this application. The Risk assessment chapter (Ch 2) then focuses on identifying and characterising risks to the health and safety of people or to the environment from dealings with the conventionally bred InVigor® x Roundup Ready® canola. The potential for harm due to expression of the introduced genes in other related plants, including weedy species, as a result of gene transfer was assessed in Risk Scenario 4 and was not identified as a risk that warranted further assessment The potential for expression of the introduced genes to lead to increased spread and persistence of the GM canola in the environment, including agricultural settings, was assessed in Risk Scenario 2 and was not identified as a risk that warranted further assessment. Volunteer InVigor® x Roundup Ready® canola and related species can be controlled by a range of alternative herbicides, tank mixing, and non-chemical management methods.
Shire is now of the opinion that concerns regarding cross fertilisation and increased maintenance costs through inappropriate germination on Council property have been satisfactorily addressed for the purposes of these trials.	Noted.
Council expresses strong feeling that the Government should preclude the release and use of GMOs until demonstrated to be safe scientifically. GMOs should be considered as part of an integrated regional natural resource management approach.	These issues were considered in Chapter 2 of the RARMP. 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 for this release considered information provided by the applicant and currently available scientific information, and concluded that risks to human health and the environment are negligible. As required by the Act, extensive consultation with a wide range of experts, agencies and authorities, and with the public, will be undertaken before the Regulator makes a decision on this application.
Community members are concerned about potential impact on organic or bio-dynamic producers, which could also have impact on regional economy. Potential damage to 'clean and green' image and impact on livelihoods. Many local organic and bio- dynamic farms in the region. Potential of damage to markets if consumers in other countries reject GM crops.	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. 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 are matters for States and Territories, and industry.
Council strongly supports the current moratorium in SA.	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. Some areas may be designated under State or Territory law for the purpose of preserving the identity of GM or non-GM crops (or both) for marketing purposes. However, marketing issues are outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence.

Summary of issues raised	Comment
Recognises that the commercial cultivation of GM canola is still subject to restriction in SA but region borders VIC which does not have such restrictions. 'Clean green' reputation and market may be affected as a result of real or perceived presence of GM canola in the area, given the proximity to VIC. Noted that issues of marketability and trade are outside evaluation scope. However, is concerned about loss of economic benefits and marketability due to commercial release of GM canola.	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. 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 are matters for States and Territories, and industry.
Considers there is insufficient research and evidence into possible deleterious environmental and public health issues to warrant the issue of a licence.	This issue was considered in Chapter 2 of the RARMP. The RARMP for this release considered information provided by the applicant and currently available scientific information, and concluded that risks to human health and the environment are negligible.
Would like to encourage the Regulator to employ the precautionary principle when preparing RARMP.	This issue was considered in Chapter 2 of the RARMP. The RARMP for this release considered information provided by the applicant and currently available scientific information, and concluded that risks to human health and the environment are negligible.
Has not identified any specific risks to human health and safety and the environment that should be considered in the RARMP.	Noted.
Note that some medicines may contain canola oil as an ingredient. Asks this to be considered.	Toxicity of the parental GM canola lines to humans is considered in Chapter 1, Section 5.4.1. The conventionally bred GM canola proposed for release is not expected to be any more toxic or allergenic than the parental lines as the same genes will be expressed. The proteins encoded by the introduced genes are well characterised and are not known to be toxic or allergenic. The potential for allergic reactions in people, or toxicity in people and other organisms, as a result of exposure to GM plant materials was assessed in Risk Scenario 1 and was not identified as a risk that warranted further assessment. FSANZ is responsible for human food safety assessment and food labelling, including GM food. Products derived from InVigor® x Roundup Ready® are approved by FSANZ for use in human food.
The applicant needs to consider both crop management plans (Roundup Ready® and InVigor® canola) simultaneously but should submit only one management plan for the release of the stacked GM canola. That plan should include the mandatory audit process from the InVigor® canola crop management plan.	This issue was considered in Chapter 1 of the RARMP. Crop Management Plans have been developed separately by Bayer CropScience and Monsanto for InVigor® and Roundup Ready® canola, respectively. The applicant has stated that growers are required to follow these CMPs when growing either InVigor® canola, Roundup Ready® canola or InVigor® x Roundup Ready® canola.

Summary of issues raised	Comment
It is not clear if there is an available strip test for the presence of InVigor traits. A strip test would streamline identification and management of volunteers.	 This issue was addressed in Chapter 4 of the RARMP (ie proposed licence conditions). ELISA (Enzyme Linked ImmunoSorbent Assay) test strips have been developed to act as a rapid and accurate way of detecting InVigor® hybrid and Roundup Ready® canola separately. Used in conjunction, the applicant expects that these test strips will be used in Australia to positively identify the stacked event if InVigor® x Roundup Ready® canola is commercially released. The proposed licence conditions include a requirement to provide a testing methodology to identify the presence of the GMO or genetic material in a recipient organism within 30 days of the licence being issued.
There needs to be a full report provided from trial under licence DIR104 at the conclusion of the trial (Feb 2014). The report should include all relevant safety- related data for assessment from these dealings before further consideration of DIR 108 application for commercial release.	The RARMP for this release considered information provided by the applicant and currently available scientific information, and concluded that risks to human health and the environment are negligible.
Currently considering declaring the Shire as GM free. Council officers have concerns that approval of unrestricted licence to release GM canola would fail to take into account locally significant risks to health, safety and the environment. Encourage consideration of working within local areas to determine the appropriateness or otherwise of GM crops.	These issues were considered in Chapter 2 of the RARMP. 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. Some areas may be designated under State or Territory law for the purpose of preserving the identity of GM or non-GM crops (or both) for marketing purposes. However, marketing issues are outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence The RARMP for this release considered information provided by the applicant and currently available scientific information, and concluded that risks to human health and the environment are negligible.
Notes that that there have been no reports of adverse environmental effects from either field trial or commercial release of the GM canolas (stacked or unstacked). Considers there are no new environmental concerns to be incorporated in the RARMP.	Noted.
Advises the OGTR that Shire Council has no position on the release of modified canola and does not wish to comment.	Noted.
Acknowledges that specific locations for the release have not been determined at this stage. No advice to provide at this preliminary phase, however its position is that it will adopt the precautionary principle in this matter and therefore opposes any release of GM canola within its Shire in the future due to uncertainties and potential impacts of GMOs on health, environment and agriculture in the district.	These issues were considered in Chapter 2 of the RARMP. Application DIR 108 is for the commercial release of GM canola in all commercial canola growing areas of Australia. The RARMP for this release considered information provided by the applicant and currently available scientific information, and concluded that risks to human health and the environment are negligible. 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. 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 are matters for States and Territories, and industry.

Summary of issues raised	Comment
 Council policy is that it strongly prefers the district to be GMO free. Does not support issuing of a licence to Bayer for dealings involving the commercial release of GM InVigor x Roundup Ready canola. Before commercial release is allowed in SA, Council believes the following concerns need to be addressed: commercial impact on overseas market assurance that effective segregation will be available a caveat requiring companies to make good any economic loss incurred by farmers and businesses from 	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. 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 are matters for States and Territories, and industry. Application DIR 108 is for the commercial release of GM canola in all commercial canola growing areas of Australia. The RARMP for this release concluded that risks to human health and the environment are negligible. Therefore, the Regulator has not proposed any limits or controls to restrict the release.
 unintended consequences of the release. If trials of GMOs are to occur, the company performing the trial should: notify council of the sites of those trials advise all neighbouring farmers with properties are within 3km of site advise apiarists with bees within 3km of site 	
 ensure harvesting and carriage of seed produced is controlled to prevent escape of seed. 	
Recognises that the commercial cultivation of GM canola is still subject to restriction in SA but region borders VIC which does not have such restrictions. Noted that issues of marketability and trade are outside evaluation scope. However, is concerned about loss of economic benefits and marketability due to commercial release of GM canola. 'Clean green' reputation and market may be affected as a result of real or perceived of GM canola into the area, given the proximity to VIC.	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. 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 are matters for States and Territories, and industry.
Considers there is insufficient research and evidence into possible deleterious environmental and public health issues to warrant the issue of a licence.	This issue was considered in Chapter 2 of the RARMP. The RARMP for this release considered information provided by the applicant and currently available scientific information, and concluded that risks to human health and the environment are negligible.
LGA is sure that the representatives of the local agricultural sector would be interested in the proposal of the release of genetically modified canola into the commercial growing areas of Australia.	A public invitation to comment on the RARMP will be published in a national newspaper, regional newspapers, the Commonwealth Gazette and on the OGTR website, as well as sent to people and organisations that have registered on the OGTR mailing list.

Appendix B Summary of issues raised in submissions received from prescribed experts, agencies and authorities on the consultation RARMP for DIR 108

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

Summary of issues raised	Comment
The Regulator should consider amending the RARMP to outline alternative measures to manage volunteers of the GM canola.	The RARMP has been modified to provide further information on the herbicide groups available to control the GM canola.
LGA has no policies on the growing or use of GM crops. Considers such issues are best handled through the relevant government agencies and notes that the West Australian Local Government Association is being consulted.	Noted.
Council has developed a Roadside Native Vegetation Plan. Concerned that road reserves in cropping areas will be at risk with the planting of GM crops, particularly in respect to the use of any chemical. Strongly opposes gene technology and has endorsed this policy since Council's inconting (1 January 1007)	The potential for expression of the introduced genes to lead to increased spread and persistence of the GM canola in the environment, including non- cropped disturbed habitats such as roadsides, was assessed in Risk Scenario 2 and was not identified as a risk that could be greater than negligible. Further information on the control of volunteer GM canola on roadsides can be found in the <i>Control of roadside canola volunteers</i> fact sheet, available on the OCTR website.
Council does not wish to make a submission.	Noted.
Supportive of the assessment that the proposed release poses low risk to the health and safety of people or the environment as a result of gene technology. Notes that GM InVigor® canola and GM Roundup Ready® canola were individually approved in 2003 for commercial release and no information has arisen to indicate these licences should be varied, suspended or cancelled. Understands that if a licence were to be issued, general conditions are proposed to cancel the set of	Noted.
ensure that there is ongoing oversight of the GM canola.	
Council has no formal comment to make of Application DIR 108, however requests the Regulator ensure that prior to release the proposal will not have any detrimental impacts upon the existing canola industry.	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. 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 are matters for States and Territories, and industry.

Summary of issues raised	Comment
Main concern is the potential for any encroachment of GM canola onto neighbouring land and thereby becoming an environmental weed with unknown environmental impacts which could increase over time. Notes the RARMP concludes that the risks from the proposed release of GM canola are assessed to be negligible. States that this however does not negate the need for due diligence in the release of GM canola in greater quantities and in an uncontrolled environment.	The potential for expression of the introduced genes to lead to increased spread and persistence of the GM canola in the environment was assessed in the context of a commercial scale release in Risk Scenario 2 and was not identified as a risk that could be greater than negligible.
The potential for new, emerging or environmental weeds to have significant, long term, negative effects on social, economic and environmental outcomes needs to be recognised and acted upon at the local government level. LGA has a duty of care to ensure that all measures of monitoring and control are adhered to. Requests that, If the application is approved, a rigorous monitoring and reporting regime is applied and that LGAs are kept informed of the results of this monitoring.	As the risks to the health and safety of people or the environment from the proposed dealings are assessed to be negligible, no specific risk treatment measures are proposed. However, general licence conditions are proposed to ensure that there is ongoing oversight of the release. These include a requirement to submit an annual report containing information about the volumes of the GMOs grown in each State. Any adverse impacts or new information relating to risks to human health and safety or the environment caused by the GMOs must also be promptly reported to the Regulator. The proposed licence also includes conditions relating to post release review (see Chapter 3, Section 4) that require the licence holder, upon request by the Regulator, to collect and provide further information on the progress of the dealing.
Requests that the Central Highlands Agribusiness Forum be informed of this application.	All of the authorities listed as sponsors of the Central Highlands Agribusiness Forum on their website have been consulted twice on application DIR 108. In addition, a public invitation to comment on the RARMP was published in a national newspaper, regional newspapers, the Commonwealth Gazette and on the OGTR website, as well as sent to people and organisations that have registered on the OGTR mailing list.
Acknowledges that specific locations for the release have not been determined at this stage. Council resolved to adopt the precautionary principle and advise the OGTR that it opposes the release of this GM canola within the Shire due to the uncertainties and potential impact of GMOs on health, environment and agriculture in the Shire district.	Application DIR 108 is for the commercial release of GM canola throughout Australia. The RARMP for this release considered information provided by the applicant and currently available scientific information, and concluded that risks to human health and the environment are negligible.
Agrees with the Regulator's assessment of this application. Notes in regard to potential synergistic effects of gene stacking that all the proteins encoded by the introduced genes operate through independent biochemical pathways.	Noted.
The RARMP for DIR 108 notes that FSANZ has already assessed and approved the use of food derived from the individual parent lines for human consumption. These approvals also cover the hybrid GM canola of this licence.	Noted.

Summary of issues raised	Comment
Considers that the OGTR would take into account all relevant factors when preparing a RARMP. Given the nature of the organism, the purpose of the release and the previous releases approved by the Regulator, considers that the proposed licence conditions are appropriate. Accordingly, has no concerns regarding the release.	Noted.
Have no comments on the RARMP and supports the Regulator's conclusion that the proposed release of GM canola into the environment poses negligible risks to the health and safety of people or the environment.	Noted.
Satisfied with the conclusions of the consultation RARMP.	Noted.

Appendix C Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 108

The Regulator received nine submissions from the public on the consultation RARMP. These submissions, summarised in the table below, raised issues relating to human health and safety and the environment. These 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.

View (general tone): n = neutral; x = do not support; y = support

Type: A: Agricultural/Industry organisation; I: Individual; NGO: Non-government organisation.

Issues raised: Ag: Agricultural practices; AS: Applicant suitability; B: Benefits of gene technology; cp: Consultation process; E: Environment; H: Human health; hu: Herbicide use; I: Information; RA: Risk analysis; Res: further research; W: Weediness.

Other abbreviations: Act: *Gene Technology Act 2000*; APVMA: Australian Pesticides and Veterinary Medicines Authority; Ch: Chapter; FSANZ: Food Standards Australia New Zealand; GM: Genetically Modified; GMO: Genetically Modified Organism; LC: Licence Conditions; RARMP: Risk Assessment and Risk Management Plan.

Sub. No:	Туре	View	Issue	Summary of issues raised	Comment		
1	I	У	Ag	Believes this technology has excellent potential for improving the sustainability of our farming systems. Stacking of herbicide resistance will help reduce reliance on glyphosate and thereby reduce herbicide resistance pressure. Strongly recommends the release of stacked herbicide tolerance into Australian farming systems.	Noted. Benefits of gene technology are outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence.		
2	I	x	H, E	Considers that gene technology to enable increased use of glyphosate and glufosinate ammonium could be a threat to the environment and so to human health.	The RARMP for this release considered information provided by the applicant and the currently available scientific information, in the context of the large scale of the proposed release, and concluded that risks to human health and the environment are negligible. The APVMA has regulatory responsibility for the registration of agricultural chemicals, including herbicides, in Australia.		
Sub. No:	Туре	View	Issue	Summary of issues raised	Comment		
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3	I	X	Н	Does not wish family to consume GM products. Feels that there is no protection from the inclusion of GM products in food and feed. Requests that the Department of Health and Ageing regulates gene technology by not issuing a licence to release these GM canola plants.	The RARMP for this release considered information provided by the applicant and the currently available scientific information, in the context of the large scale of the proposed release, and concluded that risks to human health and the environment are negligible. The potential for allergic reactions in people, or toxicity in people and other organisms, as a result of exposure to GM plant materials was assessed in Risk Scenario 1 and was not identified as a risk that could be greater than negligible. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has approved the use of food derived from GM InVigor® canola and GM Roundup Ready® canola for human consumption. These approvals also cover GM InVigor® x Roundup Ready® canola.		
4	I	X	Η	The canola absorbs the Roundup herbicide and harbours it for human consumption. By changing the genes in canola we are gambling with our food supply and our health.	The RARMP for this release considered information provided by the applicant and the currently available scientific information, in the context of the large scale of the proposed release, and concluded that risks to human health and the environment are negligible. FSANZ is responsible for human food safety assessment and food labelling, including GM food.		
5	Ι	I x	I X	I x H,	H, E	Opposed to GMOs. There is not long enough between the development and release of GM crops for long term effects on other plants, microorganisms and animals to be known.	The RARMP for this release considered information provided by the applicant and the currently available scientific information, in the context of the large scale of the proposed release, and concluded that risks to human health and the environment are negligible.
			hu	Does not like the idea of chemical resistant food as it allows people to use the chemicals without considering their effect on the environment and people. Believes no chemical that kills organisms is safe and a big enough exposure could kill people. Believes Roundup will be banned one day like DDT and other chemicals once thought to be safe. The more chemicals we allow to pollute our food and environment the less healthy the people and all other species (cites example of changing sex of crocodiles as a result of chemical runoff).	Issues relating to herbicide use are outside the scope of the Regulator's assessments. The APVMA has regulatory responsibility for the registration of agricultural chemicals, including herbicides, in Australia.		

Sub. No:	Туре	View	Issue	Summary of issues raised	Comment
			В	We have enough grain already produced in Australia. The only long term benefit would be to chemical companies.	Benefits of gene technology are outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence.
			ср	Most people will not get to hear about this proposal. Have the Aboriginal population been consulted?	The Act requires extensive consultation on all DIR RARMPs with a wide range of experts, agencies and authorities, and with the public. The public invitation to comment on the RARMP must be published in the Commonwealth Gazette, in a national newspaper and on the OGTR website. The Regulator routinely exceeds these requirements. For this commercial release application, the invitation to comment was published in regional newspapers in every Australian State and Territory in addition to the required national newspaper, and was also sent to over 600 people and organisations that have registered on the OGTR mailing list.
6	N	x	hu	Amazed that OGTR cannot see that there could be problems with growing this canola in Australia. It is bad enough that food is not adequately labelled, but to bring in something that could be twice as bad is abhorrent. Question why we rely on the testing done by the biotech companies. Suggests this provides free reign and no hurdles. Claims that glyphosate has been linked to birth defects and plant diseases and that glufosinate ammonium has been shown to cause premature birth, intra- uterine death and abortion in rats (links to web sites provided).	The RARMP for this release considered 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, as well as information in the application, and concluded that risks to human health and the environment are negligible. Issues relating to herbicide use are outside the scope of the Regulator's assessments. The APVMA has regulatory responsibility for the registration of agricultural chemicals, including herbicides, in Australia.
7		x	hu	Objects to Bayer growing GM canola in Australia. Glyphosate is linked to birth defects and plant diseases and glufosinate ammonium is reprotoxic. There are alternatives and ongoing long term health is paramount.	Issues relating to herbicide use are outside the scope of the Regulator's assessments. The APVMA has regulatory responsibility for the registration of agricultural chemicals, including herbicides, in Australia.

Sub. No:	Туре	View	Issue	Summary of issues raised	Comment
8		X	AS	Claims that Bayer CropScience is unsuitable to hold licence DIR 108 within the meaning of the Act. The Regulator must have regard to Bayer's history of law breaking and non- compliance around the world over the past 10 years, as it applies to human health, safety and the environment. In support of this contention, a number of reports of international events associated with the activities of the Bayer group were listed. Asks that information provided in the application regarding suitability of the applicant be published.	The Regulator has assessed the suitability of Bayer CropScience Pty Ltd to hold a DIR licence as required by the Act. Bayer is considered suitable as the Regulator is satisfied that no relevant convictions have been recorded, no licences or permits have been cancelled or suspended under laws relating to the health and safety of people or the environment, and the organisation has the capacity to meet the conditions of the licence.
			W, RA	The Regulator and Bayer use out of date evidence when more recent literature are not cited. Case is based on selective, partial and out of date evidence. Asks that Bayer be required to update the evidence supporting its application to include recent commercial experience and scientific research published in the past decade. Evidence on canola weediness cited is from 2002 (six years before GM canola was first grown in Australia). No new evidence on the environmental impacts of GM canola in Australia have been collected. The out-of-datedness of the environmental information is shown, for example, by the referencing of Monsanto report 0118/1 from 2001.	The RARMP for this release considered information provided by the applicant and the currently available scientific information (including many recent as well as relevant older publications and reports), in the context of the large scale of the proposed release, and concluded that risks to human health and the environment are negligible. Canola is not currently listed as being a noxious weed in any State or Territory in Australia. The potential for the introduced genes to increase the spread and persistence of the GM canola was assessed in Risk Scenario 2 (Chapter 2). Updated information on weediness of the GM parental canola lines is also provided in Chapter 1 Section 5.5. It was concluded that: the introduced genes are unlikely to alter the response of the GM canola to biotic and abiotic stresses that naturally limit the geographical distribution of the parent species; the genetic modifications would only confer a selective advantage in managed environments in which the corresponding herbicide(s) were used; and canola plants with tolerance to both glufosinate ammonium and glyphosate can be controlled by other herbicides or mechanical means.

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			RA	 RARMP cites unpublished reports from Bayer and other such as Dr Ian Heap, who has BASF logos emblazoned on the pages RARMP does not discuss recent literature such as: Schafer et al 2011 (on the spread of canola in North Dakota) Brimner et al (on the influence of herbicide tolerant canola on the environmental impact of weed management) Zhang et al 2011 (on plant miRNAs in food) Arisa et al 2011 (who found Bt toxin residues in pregnant women and their fetuses) ABC radio national health report (on the role of canola in macular degeneration) 	 The RARMP for this release considered information provided by the applicant and the currently available scientific information, in the context of the large scale of the proposed release, and concluded that risks to human health and the environment are negligible. <i>Specific responses:</i> Schafer et al 2011 was published subsequent to preparation of the consultation RARMP. It has been cited in the final RARMP. Brimner et al 2004 relates to herbicide use, which is the responsibility of the APVMA. Zhang et al 2011 was published subsequent to preparation of the consultation RARMP. It demonstrates that exogenous plant microRNA (miRNA) can regulate the expression of target genes in mammals. GM InVigor® x Roundup Ready® canola is not modified to express novel miRNAs. FSANZ is responsible for human food safety assessment and food labelling, including GM food. Aris & Leblanc (2011) report evidence of foetal exposure to Bt proteins through maternal blood transmission. GM InVigor® x Roundup Ready® canola does not contain Bt proteins. Food safety is the regulatory responsibility of FSANZ, who have provided a public response to this paper via a fact sheet published on the FSANZ website. ABC Radio National report (2004) – The genetic modifications to the canola considered in the application are not part of the fatty acid production pathways, and the published nutritional tests of GM InVigor® x Roundup Ready® canola (summarized in Section 6.2.4 of the RARMP) indicate that the levels and composition of fatty acids are within the range of existing commercial canola varieties. If any link between vegetable fat consumption and macular degeneration did exist, the GM canola poses no greater risk than non-GM canola varieties.

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			RA	The Regulator should apply the Precautionary Principle, as enunciated in the Act and in the Environment Protection and Biodiversity Conservation Act. The OGTR has no benchmark, standards or other objective criteria by which to assess the evidence presented. Thus, objective, science-based decisions are impossible under the OGTR system and every decision is ad hoc.	The relevant provision of the Act (Section 4(aa)) applies when there are threats of serious or irreversible environmental damage. To date, the Regulator has not identified such a situation in the applications assessed. The precautionary approach adopted by the Regulator is outlined in the <i>Risk Analysis Framework</i> document available on the OGTR website.
			W, Res	The Regulator has not required any systematic environmental data collection since the commercial release of Roundup Ready® canola began in 2008. In the absence of this data, the OGTR assumes that the planting of GM canola creates no new issues when grown and transported, where it is frequently spilled on roadsides and into other disturbed environments. Roundup Ready® canola has led to contamination of roadsides, non-GM farmers and other disturbed environments in Australia. Stacked GM canola tolerant to Liberty and Roundup Ready® herbicides may be a greater problem weed than either non- GM canola or GM canola tolerant to a single herbicide. The Regulator has commissioned no research that would validate or disprove assumptions of negligible outcrossing and impacts on natural environments. Asks that the Regulator require such research to be conducted and evaluated before issuing any further GM canola licences.	The potential for expression of the introduced genes to lead to increased spread and persistence of the GM canola in the environment, including non-cropped disturbed habitats such as roadsides, was assessed in Risk Scenario 2 and was not identified as a risk that could be greater than negligible. Updated information on weediness of the GM parental canola lines is also provided in Chapter 1 Section 5.5. The risk assessment concluded that there are negligible risks to people and the environment from the proposed release of GM canola. Therefore, no specific licence conditions are imposed to treat these negligible risks. However, general conditions are proposed to ensure that there is ongoing oversight of the release. These include conditions relating to post release review (see Chapter 3) that require the licence holder, upon request by the Regulator, to collect and provide further information on the progress of the dealing.

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			W	The Biology document from 2002 states: canola is a problem weed in agricultural areas due to high seed losses and a persistent seedbank; canola is a plant which occurs in disturbed habitats such as roadsides, railway verges and field margins; seed can persist for up to 10 years, and; canola outcrossing rates between 12 – 47% can occur. But the 2011 updated document states: there are limited data on outcrossing rates under Australian conditions, and; the maximum outcrossing rate of 0.197% was measured at 1.5 km.	 The quotes from the 2011 Biology document are referring to a specific publication and accurately report its findings. The 2011 biology document also states: (a) Brassica napus is a classified as a category 2 weed in natural ecosystems and category 5 weed in agricultural ecosystems (pg 28) (b) Large seed banks of canola can build up in the soil as a result of high amounts of seed loss before and during harvest (pg 19) (c) Populations of canola can be found on roadside verges, in field margins and along railway lines in all countries where it is grown (pg 29) (d) Studies in the Northern Hemisphere suggest that viable seeds of canola may persist in disturbed soils for at least 5 years and possibly up to 16 years in undisturbed soil (pg 18) (e) Based on the seven references cited in Beckie et al (2003) average outcrossing between adjacent plants would be approximately 30%, but rates up to 55% have been recorded (pg 30).
				The Regulator ignores impediments to independent research posed by the GM industry prohibitions on access to GM varieties for research purposes. GM companies also censor any negative results from research as disclosed in Nature Biotechnology "Under Wraps" and Scientific American "A Seedy Practice". The Regulator should require key additional scientific data to address this. The Regulator has a responsibility to ensure that all evidence is available and published.	The RARMP for this release considered information provided by the applicant and the currently available scientific information, in the context of the large scale of the proposed release, and concluded that risks to human health and the environment are negligible. Unless a declaration of commercial confidential information (CCI) is made, all information submitted by an applicant is available to the public upon request. RARMPs for all DIR applications are made publicly available on the OGTR website. The Regulator invited people widely to comment on the RARMP for DIR 108 via newspaper advertisements and on the OGTR website. The invitations to comment indicated how copies of the RARMP and other documents including the application could be obtained

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			RA	The Regulator has not prepared a RARMP, but has created five scenarios that exclude the worst cases and ignored evidence of harm. The RARMP frames the simplified scenarios then dismisses them. This 'straw man' approach cannot lead to the development of robust RARMPs. The RARMP is not objective, logical or transparent. GM crops are declared to be safe based on assumptions that are rarely testable, verifiable or refutable. The RARMP is not a scientific document and uses the word 'significant' on numerous occasions although only a handful of these relate to statistical significance. The RARMP does not quantify the risks that it dismisses as negligible. Yet these claims of negligible risk are the basis for recommending approval of application DIR 108.	RARMPs are prepared using the risk analysis model and terminology as described in the Regulator's Risk Analysis Framework (RAF), which is based on the internationally recognised Australia-New Zealand Standard on Risk Management (AS/NZS 4360:2004). Risks are assessed within the risk assessment context, and a risk is only identified for further assessment when a risk scenario is 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. The RARMP for this release considered information provided by the applicant and the currently available scientific information. Five risk scenarios were postulated in the RARMP. The characterisation of these risk scenarios in relation to both the seriousness and likelihood of harm did not identify any risks that could be greater than negligible. The term 'significant risk' is used in the Act. As indicated in the footnotes in the consultation RARMP, if the Regulator considers that none of the proposed dealings pose a significant risk to people or the environment, section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on a RARMP. However, the Regulator allowed up to 8 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.
			St	The Regulator assumes that combining the traits approved under licences DIR 020 and DIR 021 poses no additional risks or hazards. Disagrees with the Regulator's assumption that, as the individual genetic events now stacked in the one plant were individually assessed as safe, further assessment is unnecessary.	The RARMP for this release considered information provided by the applicant and the currently available scientific information, in the context of the large scale of the proposed release, and concluded that risks to human health and the environment are negligible.

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9	A	у	None	 Support the commercial release of the GM canola. Make the following comments: 1. Both InVigor® and Roundup Ready® canola systems have already been approved by the Regulator. 2. Roundup Ready® canola has been a success in Australia. 3. InVigor® and Roundup Ready® canola command a significant market share in Canada, with no adverse impaces on human health or the environment reported. 4. Australian farmers require additional herbicide options for use in rotations to minimise weed resistance. 	The Regulator does not consider benefits as this is outside the scope of assessment required by the Act.