



**Australian Government**

**Department of Health and Ageing**

**Office of the Gene Technology Regulator**

**Risk Assessment and  
Risk Management Plan for  
DIR 103**

Limited and controlled release of canola genetically  
modified for enhanced yield and delayed leaf senescence

Applicant: DPI Victoria

August 2010

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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 103 from the Victorian Department of Primary Industries (DPI Victoria). The licence authorises dealings involving the limited and controlled release of up to 10 lines<sup>1</sup> 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 public<sup>2</sup>.

## **The application**

DPI Victoria has applied for a licence for dealings involving the intentional release of up to 10 lines of GM canola on a limited scale and under controlled conditions. The GM canola lines have been genetically modified for enhanced yield and delayed leaf senescence. The trial may take place at two sites at Victorian government research stations in the local government areas of Horsham and Southern Grampians, on a maximum combined area of 0.8 ha per growing season, between the date of issue of the licence and May 2012. The applicant proposed planting in both summer and winter growing seasons.

The purpose of the trial is to conduct experiments to evaluate agronomic performance, including seed yield, of the GM canola lines under field conditions. Some seed would be collected and retained for analysis and possible future trials, subject to further approval(s). Material from the GM canola will not be used in human food or animal feed.

The applicant intends to release GM canola modified to contain a gene derived from a common soil bacterium. Expression of the gene is expected to enhance yield and delay leaf senescence in the GM canola plants. The GM canola lines also contain an antibiotic resistance gene which was used to identify transformed plants during initial development of the GM plants in the laboratory.

DPI Victoria proposed a number of controls to restrict the spread and persistence of the GM canola lines and the introduced genetic materials in the environment that were considered during the evaluation of the application.

## **Confidential Commercial Information**

Some details, including unpublished data from glasshouse experiments, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

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<sup>1</sup> The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification resulting from a single transformation event.

<sup>2</sup> More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/>>), and in the Regulator's *Risk Analysis Framework* (OGTR 2009) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

## **Risk assessment**

The risk assessment took into account information in the application (including proposed containment measures), previous approvals and relevant scientific/technical knowledge. 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 were postulated (risk scenarios), and these scenarios were evaluated to identify those that warrant detailed characterisation. This process is described as risk identification.

Eight 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; alter characteristics that may impact on the spread and persistence of the GM canola; or produce unintended changes in the biochemistry of the GMO. The opportunity for gene flow to other organisms, and its effects if it were to occur, was 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 eight risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not identify any risks that required further assessment.

Risks to the health and safety of people, or the environment, from the proposed release of the GM canola lines into the environment are assessed to be **negligible**. Hence, the Regulator considers that the dealings involved in this limited and controlled release **do not pose a significant risk** to either people or the environment.

## **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, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through the licence conditions.

As none of the eight risk scenarios characterised in the risk assessment give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is assessed to be **negligible**. 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. However, conditions are imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment and to limit the proposed release to the size, locations and duration requested by the applicant, as these were important considerations in establishing the context for assessing the risks.

The licence conditions require DPI Victoria to **limit** the release to a total area of 0.8 ha per growing season at two sites between the date of issue of the licence and May 2012. The **control** measures include containment provisions at the trial site; preventing the use of GM plant materials in human food or animal feed; destroying GM plant materials not required for further research or planting; transporting GM plant materials in accordance with the Regulator's transportation guidelines; and conducting post-harvest monitoring at the trial sites to ensure all GMOs are destroyed.

## ***Conclusions of the RARMP***

The risk assessment concluded that this limited and controlled release of up to 10 lines of GM canola on a maximum total area of 0.8 ha per growing season over two years in the Victorian local government areas of Horsham and Southern Grampians, 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 limit the release to the size, locations and duration proposed by the applicant, and to require controls in line with those proposed by the applicant, as these were important considerations in establishing the context for assessing the risks.

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## Abbreviations

the Act	<i>Gene Technology Act 2000</i>
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
CaMV	Cauliflower Mosaic Virus
CCI	Confidential Commercial Information as declared under section 185 of the <i>Gene Technology Act 2000</i>
DIR	Dealings Involving intentional Release
DNA	Deoxyribonucleic Acid
DPI Victoria	The Victorian Department of Primary Industries
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
GM	Genetically Modified
GMAC	Genetic Manipulation Advisory Committee
GMO	Genetically Modified Organism
ha	hectare
HGT	Horizontal Gene Transfer
<i>hph</i>	hygromycin phosphotransferase gene
HPT	hygromycin phosphotransferase enzyme
<i>ipt</i>	isopentenyltransferase
IPT	Isopentenyltransferase enzyme
LGA	Local Government Area
mRNA	Messenger Ribonucleic Acid
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NLRD	Notifiable Low Risk Dealing
<i>nos</i>	nopaline synthase gene
OGTR	Office of the Gene Technology Regulator
PC2	Physical Containment level 2
PCR	Polymerase Chain Reaction
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
RNA	Ribonucleic Acid
T-DNA	Transfer DNA
TGA	Therapeutic Goods Administration
Ti	Tumour-inducing

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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 103 from the Victorian Department of Primary Industries (DPI Victoria). The licence authorises dealings involving the limited and controlled release of up to 10 lines<sup>3</sup> 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 public<sup>4</sup>.

## The application

DPI Victoria has applied for a licence for dealings involving the intentional release of up to 10 lines of GM canola on a limited scale and under controlled conditions. The GM canola lines have been genetically modified for enhanced yield and delayed leaf senescence. The trial may take place at two sites at Victorian government research stations in the local government areas of Horsham and Southern Grampians, on a maximum combined area of 0.8 ha per growing season, between the date of issue of the licence and May 2012. The applicant proposed planting in both summer and winter growing seasons.

The purpose of the trial is to conduct experiments to evaluate agronomic performance, including seed yield, of the GM canola lines under field conditions. Some seed would be collected and retained for analysis and possible future trials, subject to further approval(s). Material from the GM canola will not be used in human food or animal feed.

The applicant intends to release GM canola modified to contain the *isopentenyltransferase (ipt)* gene derived from the soil bacterium *Agrobacterium tumefaciens*. The *ipt* gene encodes the isopentenyltransferase enzyme, which is involved in cytokinin biosynthesis. Expression of the *ipt* gene is expected to enhance yield and delay leaf senescence in the GM canola plants.

The GM canola lines also contain an antibiotic resistance gene, *hph*, derived from the bacterium *Escherichia coli*. The *hph* gene encodes hygromycin phosphotransferase, which confers resistance to the antibiotic hygromycin. This was used as a selectable marker during initial development of the GM plants in the laboratory.

In addition, the GM canola lines contain short regulatory elements to control expression of the introduced genes. These sequences are derived from *Arabidopsis thaliana*, *A. tumefaciens* and Cauliflower mosaic virus.

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<sup>3</sup> The term ‘line’ is used to denote plants derived from a single plant containing a specific genetic modification resulting from a single transformation event.

<sup>4</sup> More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030 or at <<http://www.ogtr.gov.au/>>), and in the Regulator’s *Risk Analysis Framework* (OGTR 2009) at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

DPI Victoria proposed a number of controls to restrict the spread and persistence of the GM canola lines and their genetic material into the environment. These controls were considered during the evaluation of the application.

### **Confidential Commercial Information**

Some details, including unpublished data from glasshouse experiments, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

### **Risk assessment**

The risk assessment took into account information in the application (including proposed containment measures), previous approvals and relevant scientific/technical knowledge. 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 L. (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 were postulated (risk scenarios), and these scenarios were evaluated to identify those that warrant detailed characterisation. This process is described as risk identification.

Eight 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; alter characteristics that may impact on the spread and persistence of the GM canola; or produce unintended changes in the biochemistry of the GMO. The opportunity for gene flow to other organisms, and its effects if it were to occur, was 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 eight risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not identify any risks that required further assessment. The principal reasons for this include:

- limits on the size, locations and duration of the release proposed by DPI Victoria
- suitability of controls proposed by DPI Victoria to restrict the spread and persistence of the GM canola plants and their genetic material
- limited ability and opportunity for the GM canola plants to transfer the introduced genes to commercial canola crops or other sexually related species
- none of the GM plant materials or products will be used in human food or animal feed
- 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.

Risks to the health and safety of people, or the environment, from the proposed release of the GM canola into the environment are assessed to be **negligible**. Hence, the Regulator considers that the dealings involved in this limited and controlled release **do not pose a significant risk** to either people or the environment.

## **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, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through the licence conditions.

As none of the eight risk scenarios characterised in the risk assessment give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is assessed to be **negligible**. 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. However, conditions have been imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment and to limit the release to the size, locations and duration requested by the applicant, as these were important considerations in establishing the context for assessing the risks.

## **Licence conditions**

The Regulator has imposed a number of licence conditions, including requirements to:

- limit the release to a total area of up to 0.8 ha per growing season at two sites at Victorian Government research stations within the local government areas of Horsham and Southern Grampians, between the date of issue of the licence and May 2012
- locate the trial sites at least 50 m away from natural waterways
- surround the GM canola with a 15 m pollen trap of non-GM canola plants and a 50 m monitoring zone in which sexually compatible species are not permitted to flower
- ensure no other *Brassica* crops are grown within 400 m of the GM canola plants
- harvest the GM canola plant material separately from other crops
- clean the sites and equipment used on the sites following harvest
- apply measures to promote germination of any canola seeds that may be present in the soil after harvest, including shallow tillage
- monitor the site for at least 24 months after harvest and destroy any canola plants that may grow until no volunteers are detected for a continuous 12 month period
- destroy all GM plant material not required for further analysis or future planting
- transport material from the GMOs in accordance with the Regulator's guidelines
- not permit any GM canola plant material to be used in human food or animal feed.

## **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 be subject to regulation by other agencies that also regulate GMOs or GM products include 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)<sup>5</sup>.

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<sup>5</sup> More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

FSANZ is responsible for human food safety assessment, including GM food. As the trial involves early stage research, the applicant does not intend any material from the GM canola lines proposed for release to be used for human food. Accordingly, the applicant has not applied to FSANZ to evaluate the GM canola lines. FSANZ approval would need to be obtained before they could be sold for human food in Australia.

### ***Identification of issues to be addressed for future releases***

Additional information has been identified that may be required to assess an application for a large scale or commercial release of these GM canola lines, or to justify a reduction in containment conditions. This would include:

- additional data on the potential toxicity and allergenicity of plant materials from the GM canola lines
- additional phenotypic characterisation of the GM canola lines, in particular of traits which may contribute to weediness, including abiotic and biotic stress tolerance
- characterisation of the introduced genetic material in the plants, including copy number and expression pattern.

### ***Suitability of the applicant***

The Regulator is satisfied that DPI Victoria is suitable to hold a DIR licence as 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 RARMP***

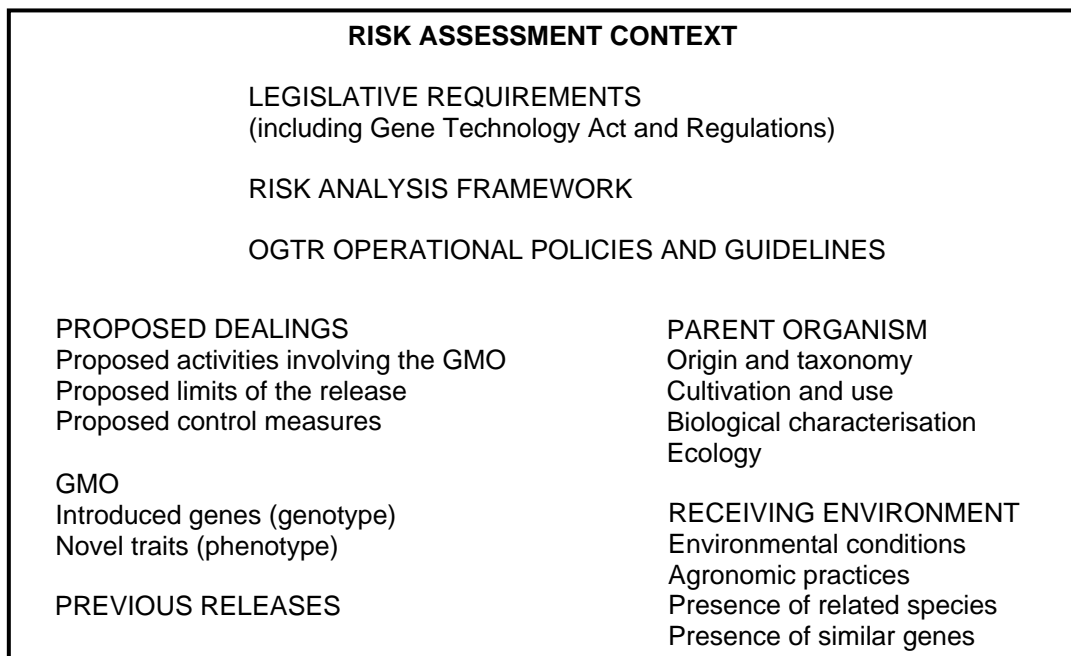
The risk assessment concluded that this proposed limited and controlled release of up to 10 lines of GM canola on a maximum total area of 0.8 ha per growing season over two years in the Victorian local government areas of Horsham and Southern Grampians, poses **negligible** risks to the health and safety 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 limit the release to the size, locations and duration proposed by the applicant, and to require controls in line with those proposed by the applicant, as these were important considerations in establishing the context for assessing the risks.

# 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).



**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 <<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)
- the genetically modified organisms (GMOs), nature and effect of the genetic modification (Section 5)
- the receiving environment (Section 6)
- previous releases of these or other GMOs relevant to this application (Section 7)

## Section 2 The legislative requirements

4. Sections 50, 50A and 51 of the *Gene Technology Act 2000* (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 Gene Technology Regulations 2001 (the Regulations) outline matters the Regulator must consider when preparing a RARMP.

5. In accordance with section 50A of the Act, the Regulator considered information provided in the application and was satisfied that its principal purpose is to enable the applicant to conduct experiments. In addition, limits have been proposed on the size, locations and duration of the release and controls have been proposed by the applicant to restrict the spread and persistence of the

GMOs and their genetic material in the environment. Those limits and controls are such that the Regulator considered it appropriate not to seek the advice referred to in subsection 50(3) of the Act. Therefore, this application is considered to be a limited and controlled release and the Regulator has prepared a RARMP for this application.

6. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee (GTTAC), Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, local council(s) where the release is proposed to take place, and the public. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. One submission was received from the public and its consideration is summarised in Appendix B.

7. Section 52(2)(ba) of the Act requires the Regulator to decide whether one or more of the proposed dealings may pose a ‘significant risk’ to the health and safety of people or to the environment, which then determines the length of the consultation period as specified in section 52(2)(d). The decision is provided in Section 3 of Chapter 2.

### **Section 3 The proposed dealings**

8. The Victorian Department of Primary Industries (DPI Victoria) proposes to release up to 10 lines<sup>6</sup> of GM canola, which have been genetically modified (GM) to enhance yield and delay leaf senescence, into the environment under limited and controlled conditions.

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

- conducting experiments with the GMOs
- breeding the GMOs
- propagating, growing, raising or culturing the GMOs
- transporting the GMOs
- disposing of the GMOs
- possession, supply or use of the GMOs for the purposes of any of the above.

10. These dealings are detailed further throughout the remainder of the current Chapter.

11. Some details, including unpublished data from glasshouse experiments, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. This information was considered during the preparation of the RARMP and was made available to the prescribed expert groups and authorities that were consulted.

#### **3.1 The proposed activities**

12. The applicant has stated that the purpose of the trial is to conduct experiments to evaluate the agronomic performance, including seed yield, of the GM canola lines under field conditions.

13. Seed from the GM canola lines proposed for release would be transported from DPI Victoria facilities in Horsham to the proposed trial sites and planted in six replications at each site in plots of approximately 10 m<sup>2</sup>. Plots would contain GM canola lines that have shown enhanced yield during glasshouse screens as well as the corresponding non-GM control for each line and a small number of other non-GM canola varieties.

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<sup>6</sup> The term ‘line’ is used to denote plants derived from a single plant containing a specific genetic modification resulting from a single transformation event.

14. At the end of the trial, plant material, including seed, would be harvested and transported to contained facilities for analysis. Any plant material not required for future trials or experiments would be destroyed. Plant materials from the GM canola will not be used for either human or animal consumption.

### **3.2 The proposed limits of the dealings (size, locations and duration)**

15. The release is proposed to take place at two Victorian government research stations in the local government areas (LGAs) of Horsham and Southern Grampians, on a maximum area of 0.8 ha per growing season between May 2010 and May 2012.

16. Only trained and authorised staff will be permitted access to the proposed locations.

### **3.3 The proposed controls to restrict the spread and persistence of the GMOs and their genetic material in the environment**

17. The applicant has proposed a number of controls to restrict the spread and persistence of the GM canola lines and the introduced genetic material in the environment including:

- locating the trial sites at least 50 m away from natural waterways
- surrounding the GM canola with a 15 m pollen trap of non-GM canola and a 50 m monitoring zone that is kept free of canola and related species
- locating the trial sites at least 400 m away from any Brassica crop
- harvesting all GM canola plants in a manner to minimise the loss of seed
- destroying all GM plant material not required for testing or future trials
- incorporating any plant material remaining at the site after harvest into the soil and allowing it to decompose
- cleaning all equipment on site
- promoting the germination of any residual seed following harvest through light tillage, irrigation, and other agronomic practices
- post harvest monitoring of the trial site on a monthly basis for 24 months and destroying any volunteer canola plants
- not permitting any GM canola plant material to be used in human food or animal feed
- transporting and storing GM plant materials in accordance with Regulator's guidelines.

18. These controls, and the limits outlined in Section 3.2, have been taken into account in establishing the risk assessment context (this chapter), and their suitability for containing the proposed release is evaluated in Chapter 3, Section 4.1.1.

## **Section 4 The parent organism**

19. The parent organism is *Brassica napus* L., which is commonly known as canola, rapeseed or oilseed rape. The term canola refers to varieties that meet specific standards on the level of toxicants (see Chapter 2, Risk scenario 1).

20. 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 2008).

21. The GM canola lines proposed for release were produced by genetic modification of plants of the advanced breeding line RR014. RR014 is not grown commercially but has been used for crossing and genetic modification work.

## **Section 5 The GMOs, nature and effect of the genetic modification**

### **5.1 Introduction to the GMOs**

22. The GM canola lines contain the *ipt* gene (GenBank accession number AE007871.2) which is expected to enhance yield and delay leaf senescence in the GM canola plants. The *ipt* gene is derived from the bacterium *Agrobacterium tumefaciens* and encodes the cytokinin biosynthesis enzyme isopentenyl transferase.

23. The GM canola plants also contain the antibiotic resistance selectable marker gene *hph*, encoding hygromycin phosphotransferase. This gene is derived from the common gut bacterium *Escherichia coli*, and confers resistance to the antibiotic hygromycin on the GM plants. The *hph* gene was used as a selective marker during early stages of development of the GM canola plants in the laboratory.

24. Short regulatory sequences (promoters and transcription termination sequences) that control expression of the introduced genes are also present in the GM canola lines. These sequences are derived from *A. tumefaciens*, the plant *Arabidopsis thaliana* (thale cress) and the plant virus Cauliflower mosaic virus (CaMV) (see Sections 5.3 and Table 1).

### **5.2 The introduced genes, their encoded proteins and end products**

#### **5.2.1 The introduced gene for enhanced yield and delayed leaf senescence, and its protein**

25. Isopentenyl transferase (IPT) is an enzyme that catalyses the first and rate-limiting step of cytokinin biosynthesis. Cytokinins are plant hormones that are involved in a wide variety of plant growth and development processes, including seed germination, flowering, seed and fruit development, plant defence responses, response to drought, nutritional signalling, control of root/shoot balance and the senescence of leaves and other plant tissues (Smart 1994; Chandless 2001; Kamada-Nobusada & Sakakibara 2009).

26. Genes encoding IPTs are present in higher plants and in some phytopathogenic microorganisms (Kamada-Nobusada & Sakakibara 2009). In rice, eight *ipt* genes involved in cytokinin biosynthesis have been identified. Similarly, seven *ipt* genes have been identified in *A. thaliana*, four of which localise to plastids (Kamada-Nobusada & Sakakibara 2009).

27. The *ipt* gene in the GM canola lines proposed for release is derived from *Agrobacterium tumefaciens* (Barry et al. 1984). *A. tumefaciens* is a common soil bacterium capable of causing tumorous growths on plants known as crown galls (Van Larebeke et al. 1974). When *A. tumefaciens* infects a plant, a portion of DNA (the transfer DNA or T-DNA) present on a tumour-inducing (Ti) plasmid is introduced into the plant chromosome. The *A. tumefaciens ipt* gene is carried on the T-DNA, along with two genes involved in auxin synthesis (*iaaM* and *iaaH*). It is the overproduction of cytokinin and auxin by infected cells that results in the tumorous phenotype (Klee & Rogers 1989).

#### **5.2.2 The end products/effects associated with the introduced gene for enhanced yield and delayed leaf senescence**

28. The aim of the genetic modification is to enhance yield and delay leaf senescence in the GM canola plants. This is achieved by expression of the *A. tumefaciens ipt* gene under the control of a developmentally regulated promoter. Delayed leaf senescence as a result of increased *ipt* expression has been studied in several plants and is associated with a number of benefits to crops, including enhanced yield, increased post-harvest life and abiotic stress tolerance.

29. The same gene has been expressed in many GM plants under the control of a number of different inducible or tissue specific promoters. This has resulted in various effects including increased resistance to symptoms of bacterial infection (Barna et al. 2008), inhibition of fungal infection (Swartzberg et al. 2008), enhanced insect resistance (Smigocki et al. 1993; Smigocki et al. 2000; Jiu & Liu 2004), altered seed development (Roedel et al. 1997; Ma et al. 2008), delayed

senescence (Smart et al. 1991; Gan & Amasino 1995) and, especially when expressed constitutively, morphological changes such as stunted growth, small leaves and less developed root systems (Schmülling et al. 1989; Li et al. 1992; Bai et al. 2009). This wide range of effects likely reflects the broad spectrum of activity that cytokinins have in plants (Sakakibara 2006).

30. Changes in cytokinin levels as a result of *ipt* expression in GM grapefruit plants have been shown to affect the expression of genes implicated in the response of grapefruit to pathogen attack. GM tobacco (*Nicotiana tabacum*) plants, with the *ipt* gene regulated by a constitutive promoter or either of two inducible promoters, showed an altered hypersensitive response to bacterial infection, with altered membrane lipid concentration and increased antioxidants (Barna et al. 2008).

31. *Nicotiana plumbaginifolia* and *N. tabacum* plants expressing *ipt* under the control of a wound-inducible promoter have increased resistance to insects, with the caterpillar *Manduca sexta* consuming a lesser amount of GM plant material and nymphs of the aphid *Myzus persicae* showing delayed development after feeding on the GM plant material (Smigocki et al. 1993; Smigocki et al. 2000). This effect was attributed to an increase in secondary metabolites in the cytokinin producing *Nicotiana* plants and was not observed in tomato expressing *ipt* (Smigocki et al. 2000).

32. Seed specific expression of *ipt* has been shown to affect seed development in GM canola and GM tobacco plants. Seeds from GM tobacco plants containing *ipt* fused with a seed-specific lectin promoter were larger than from non-GM control plants, and showed an increase in soluble protein content and dry weight (Ma et al. 2008). The increase in nutrition accumulation in the GM seeds lead to an increase in seedling growth, particularly at the early stage (Ma et al. 2008).

33. Seed development was also affected in GM tobacco plants expressing the *ipt* gene under the control of a seed specific 2S albumin *AT2S1* gene promoter from *A. thaliana* (Roeckel et al. 1997). In this study, GM tobacco plants had more capsules with a lower seed weight per capsule, and showed no difference in average seed yield. Roeckel et al (1997) also introduced the *AT2S1-ipt* construct into canola plants. The resulting GM canola had more siliques than controls, but seed number per silique, seed weight and average seed yield were not significantly different when compared to controls (Roeckel et al. 1997).

34. In contrast, GM tobacco plants expressing *ipt* under the control of a senescence-specific promoter showed a 50% increase in seed yield and biomass compared to control plants (Gan & Amasino 1995). This increase was attributed in part to delayed senescence of the leaves of the GM tobacco plants. Delayed leaf senescence is one of the most common phenotypes observed in different GM plant expressing *ipt* (Smart et al. 1991; Calderini et al. 2007; Barna et al. 2008; Nguyen et al. 2008; Ma & Liu 2009; Pasquali et al. 2009; Bai et al. 2009). However, under growth conditions that do not favour transpiration, expression of *ipt* has also been shown to accelerate senescence (Li et al. 1992).

35. Many studies have used senescence specific expression of the *ipt* gene to delay senescence while avoiding the developmental abnormalities that can be caused by early expression of cytokinins. For example, GM tobacco plants expressing *ipt* using a senescence specific promoter developed normally, produced more flowers, and showed a 50% increase in dry weight and seed yield compared to control plants (Gan & Amasino 1995). GM broccoli also expressing *ipt* at senescence showed retarded post harvest floret yellowing and little or no serious morphological abnormalities (Chen et al. 2001). However, senescence specific expression of *ipt* in the vegetable crops cauliflower and lettuce, while delaying senescence, also caused several other changes, specifically smaller curd size and greater susceptibility to fungal infection in cauliflower (Nguyen et al. 2008), and a severe delay in flowering in lettuce (McCabe et al. 2001).

36. Delayed senescence has been shown to confer drought tolerance and enhanced water use efficiency on GM tobacco plants expressing *ipt* (Rivero et al. 2007). GM *A. thaliana* plants expressing *ipt* to delay flooding-induced senescence showed increased waterlogging tolerance and improved recovery after waterlogging and submergence stresses (Huynh et al. 2005). In GM tall

fescue plants expressing the *ipt* gene under the control of a maize *ubiquitin* promoter, leaf senescence was delayed under low temperature conditions (Hu et al. 2005). These plants also demonstrated increased tillering and freezing tolerance (Hu et al. 2005).

### **5.2.3 Toxicity/allergenicity of the protein/end products associated with the introduced gene for enhanced yield and delayed leaf senescence**

37. The *ipt* gene introduced into the GM canola plants was isolated from the common soil bacterium *A. tumefaciens*. Homologues of *ipt* and the encoded enzyme occur naturally in a wide range of higher plants including *Brassica rapa*, rice and maize, as well as *A. tumefaciens* and other phytopathogenic micro-organisms (Kamada-Nobusada & Sakakibara 2009). IPTs catalyse the synthesis of cytokinins, which are naturally produced in higher plants including plants widely consumed by people and animals. On this basis, humans and other organisms have a long history of exposure to the *ipt* gene, the encoded protein and its end products, via consumption of plant material. The United States Environmental Protection Agency notes that cytokinins have low acute mammalian toxicity (EPA 1995).

38. In the laboratory, wound-induced expression of *ipt* in *N. plumbaginifolia* and *N. tabacum* has been shown to inhibit consumption by insects such as *M. sexta* and delay the development of *M. persicae* nymphs (Smigocki et al. 1993). IPT is a cytokinin biosynthetic enzyme, and it is thought that insect resistance phenotypes are due to the effects of increased cytokinin on secondary metabolites (Smigocki et al. 2000). Expression of IPT in tomato does not effect insects (Smigocki et al. 2000), and no negative effects on insects have been noted in studies of a range of GM plant species expressing additional IPT, indicating that this effect is specific to *Nicotiana* species.

39. A comprehensive search of the scientific literature yielded no further information to suggest that the encoded protein is toxic or allergenic to people, or toxic to other organisms.

40. No studies on the toxicity or allergenicity of the GM canola lines and their products have been undertaken to date as the proposed trial is at an early stage. Such studies would have to be conducted if approval was sought for the GMOs or their products to be considered for human consumption in Australia.

### **5.2.4 The antibiotic resistance marker gene (*hph*) and its encoded protein**

41. The GM canola lines proposed for release contain the *hph* gene from *E. coli*, which confers resistance to the antibiotic hygromycin (Gritz & Davies 1983). The *hph* gene (also known as *hpt* and *aph4*) encodes the enzyme hygromycin phosphotransferase (HPT), which catalyses the phosphorylation of the 4-hydroxy group on the hyosamine moiety, thereby inactivating hygromycin (Rao et al. 1983) and preventing it from killing cells producing HPT. The *hph* gene was used as a selectable marker gene in the early laboratory stages of development of the plants to enable selection of plant cells containing the desired genetic modification.

42. The *hph* 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 RARMP for DIR 073/2007 (available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir073-2007>>), the use of *hph*, or other HPT encoding genes, as marker genes in GM plants has been assessed as not posing a risk to human health and safety or the environment. The HPT protein is easily digested by simulated gastric juices and the amino acid sequence contains no similarities to known allergens (Lu et al. 2007). The European Food Safety Authority concluded that inclusion of the *hph* gene in GM plants would not significantly affect the health of humans or animals (EFSA 2004).

## 5.3 The regulatory sequences

### 5.3.1 Regulatory sequences for expression of the introduced gene for enhanced yield and delayed leaf senescence

43. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. The promoter used for expression of *ipt* in the GM canola lines is a functionally active fragment of the *A. thaliana MYB32* gene (*AtMYB32*) promoter. *AtMYB32* is a member of the R2R3 *MYB* gene family coding for transcription factors (Preston et al. 2004).

44. *AtMYB32* transcript has been detected in all major organs of five week old *A. thaliana* plants (stems, roots, flowers and leaves), with the highest levels being in flowers (Preston et al. 2004). Using a reporter gene, Preston et al (2004) showed that *AtMYB32* is developmentally regulated. The *AtMYB32* promoter directed expression in anthers during the latter stages of their development, in the stigma during floral development, and in lateral root primordia during lateral root formation. Reporter gene expression in leaves generally decreased as the leaves matured (Preston et al. 2004).

45. As well as being developmentally regulated, *AtMYB32* expression has been shown to be inducible by a range of treatments. Reporter gene expression driven by the *AtMYB32* promoter was inducible in leaves and stems by wounding and in roots by the hormone auxin (Preston et al. 2004). However, reverse northern blots either failed to detect auxin induced expression of *AtMYB32* (Yanhui et al. 2006), or gave inconclusive results for auxin treatment (Kranz et al. 1998). The *AtMYB32* gene is induced by other plant hormones including cytokinin and abscisic acid, and weakly induced by stresses including salt, light, cold and drought (Kranz et al. 1998; Yanhui et al. 2006).

46. The promoter used to control expression of the *ipt* gene in the GM canola plants proposed for release is a truncated form of the *AtMYB32* promoter, with root and pollen specific motifs deleted from its sequence, referred to as *AtMYB32xs*. As a result, the *ipt* gene is not expected to be expressed in roots or pollen. The applicant has shown that the *ipt* gene under the control of the *AtMYB32xs* promoter in the GM canola plants is expressed in young leaf tissue, while more detailed expression analyses have not been performed.

47. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. The mRNA terminator for the *ipt* gene in the GM canola lines is derived from the *nopaline synthase (nos)* gene from *A. tumefaciens*. The *nos* terminator has been used in a wide variety of constructs for plant genetic modification (Reiting et al. 2007).

### 5.3.2 Regulatory sequences for the expression of the hph gene

48. The introduced *hph* gene is under the control of the 35S gene promoter from Cauliflower mosaic virus (CaMV) and the 35S mRNA termination region from CaMV.

49. Although CaMV is a plant pathogen, the regulatory sequences comprise only a small part of its total genome, and are not in themselves capable of causing disease.

## 5.4 Method of genetic modification

50. *Agrobacterium*-mediated transformation was used to generate the GM canola lines in the proposed release. As discussed in Section 5.2.1, *A. tumefaciens* introduces a segment of its Ti plasmid, the T-DNA, into infected plant cells. The T-DNA is located between specific border sequences on the Ti plasmid, and its transfer from *Agrobacterium* to plants is dependent on the virulence genes outside the T-DNA region of the Ti plasmid.

51. Disarmed *Agrobacterium* strains have been constructed specifically for plant transformation. The disarmed strains do not contain the genes responsible for the overproduction of auxin and cytokinin (*iaaM*, *iaaH* and *ipt*), which are required for tumour induction and rapid callus growth (Klee & Rogers 1989). *Agrobacterium* plasmid vectors used to transfer T-DNAs contain well characterised DNA segments required for their replication and selection in bacteria, and for transfer

of T-DNA from *Agrobacterium* and its integration into the plant cell genome (Bevan 1984; Wang et al. 1984).

52. To generate the GM canola lines in the current application, a single transformation vector was generated from a pPZP200 vector backbone (Hajdukiewicz et al. 1994) containing the *ipt* expression cassette along with the selectable marker expression cassette (Table 1). The vector was introduced into the disarmed *Agrobacterium* strain LBA4404, which was then co-cultivated with hypocotyl explants of the canola advanced breeding line RR014. Plants were regenerated in the presence of hygromycin to allow the identification of canola plants containing the introduced gene construct.

53. There has been concern in a recent publication that transfer of *A. tumefaciens* chromosomal DNA to the plant host may accompany the T-DNA integration in 0.4% of cases (Ulker et al. 2008). However, the likelihood of *A. tumefaciens* chromosomal DNA having an influence on any resulting GM plants is regarded as small given the low likelihood of plants possessing the DNA segments necessary for expression of the *A. tumefaciens* genes. *Agrobacterium*-mediated transformation has been widely used in Australia and overseas for introducing new genes into plants and is not known to cause any adverse effects on human health and safety or the environment.

**Table 1. Expression cassettes present in the vector used to generate the GM canola plants**

Expression cassette	promoter	gene	terminator
<i>ipt</i> expression cassette	<i>AtMYB32xs</i>	<i>ipt</i>	<i>nos</i>
Selectable marker expression cassette	<i>CaMV35S</i>	<i>hph</i>	<i>CaMV35S</i>

## 5.5 Characterisation of the GMOs

### 5.5.1 Stability and molecular characterisation

54. The applicant states that all gene constructs were fully sequenced prior to transformation.

55. As the project is at an early stage, full molecular characterisation of the GM canola lines has not been carried out. T<sub>1</sub> GM canola lines were obtained from primary transformants (T<sub>0</sub>) by self-pollination and were shown to contain the introduced genes by polymerase chain reaction (PCR).

56. The transgenes have been inherited as dominant Mendelian traits in subsequent generations in the glasshouse. The GM lines proposed for release have been grown in the glasshouse through to generation T<sub>3</sub> and T<sub>4</sub> and the genetic modification has been stable over all generations.

57. The genomic location of the introduced DNA has not been characterised, but each GM canola line generated from an independent transformation event is expected to have the transgenes located at different sites in the canola genome.

58. Southern hybridisation analysis was used to determine the number of copies of the transgenes present in a subset of GM canola lines. For each line tested, a single copy of each introduced gene was observed. However, the applicant states that additional lines with a range of copy number (up to 3) may be included in the proposed trial.

59. Expression levels of the introduced *ipt* gene in T<sub>2</sub> GM canola lines has been characterised by quantitative reverse transcriptase PCR and/or Northern and Southern blot hybridisation. GM canola plants with a range of expression levels would be used in the proposed trial.

### 5.5.2 Characterisation of the phenotype of the GM canola lines

60. The purpose of the proposed trial is to assess whether the expression of the introduced *ipt* gene results in increased yield and delayed leaf senescence in the GM canola plants compared with controls when grown under field conditions. The assessment will involve field evaluation combined with the measurement of traits reflecting physiological processes, including leaf senescence, plus

those that are crucial to the grower in terms of enhancing yield and yield stability, including seed production and flowering date.

61. The phenotype of the GM canola plants over several generations has been characterised in the glasshouse. Seed from T<sub>1</sub> plants displaying delayed leaf senescence characteristics under assay conditions were used to produce T<sub>2</sub> then T<sub>3</sub> plants in the glasshouse.

62. A number of T<sub>2</sub> GM canola lines were grown under glasshouse conditions to assess plant senescence progression and development. The T<sub>2</sub> plants showed an increase in relative chlorophyll content in lower leaves when compared to non-GM controls. There was no effect on root growth in the T<sub>2</sub> GM plants, but a range of leaf senescence and yield phenotypes were observed.

63. The proposed trial would be performed with T<sub>3</sub> and T<sub>4</sub> seeds from GM canola lines that showed enhanced yield during glasshouse screens. The GM lines selected are intended to provide a range of leaf senescence and yield phenotypes to allow evaluation of the impact of the introduced gene on leaf senescence and yield under field conditions.

## **Section 6 The receiving environment**

64. The receiving environment forms part of the context in which the risks associated with dealings involving the GMO 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 2009).

### **6.1 Relevant abiotic factors**

65. 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 2008).

66. The proposed sites are both under the control of DPI Victoria and are approximately 150 km apart. One site is at the DPI Victoria Plant Breeding Centre, located 7 km from the Rural City of Horsham (population approximately 19,600 at June 2008<sup>7</sup>). This area is typical of rain-fed canola production environments in Victoria.

67. The second site is an 1100 ha DPI Victoria Research Station located 11 km from the city of Hamilton in the Southern Grampians Shire (population approximately 17,400 at June 2008<sup>7</sup>). The applicant states that the relatively high rainfall in this area is favourable for the reliable establishment of the GM canola plants and the non-GM control and pollen trap plants.

68. The LGAs within which the release is proposed both have a temperate climate (as defined by the Koeppen Classification system used by the Australian Bureau of Meteorology) and are typical of rain-fed canola production environments in Australia. The rainfall and temperature statistics for the proposed release sites are given in Table 2.

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<sup>7</sup> Source: Australian Bureau of Statistics; <http://abs.gov.au/ausstats/abs@.nsf/Products/3218.0~2007-08~Main+Features~Victoria?OpenDocument#LOCALGOVERNMENTAREAPOPULATIONS>

**Table 2. Monthly temperature and rainfall statistics for the proposed release sites\***

LGA	Weather station	Mean temperature (°C)				Mean rainfall (mm)		
		Summer max	Summer min	Winter max	Winter min	Summer monthly	Winter monthly	Annual
Horsham	Horsham Polkemmet Rd	29.1	12.6	14.1	4.1	25.1	48.3	445.5
Southern Grampians	Hamilton	24.9	10.5	12.6	4.9	36.3	74.1	686.5

\*data taken from the Australian Bureau of Meteorology website (<http://www.bom.gov.au/climate/averages/>). Temperature and rainfall data are an average of at least 96 years of records.

Summer entries are averages of monthly data from December to February, and winter entries are averages of monthly data from June to August.

69. DPI Victoria proposes to locate the GM canola trial sites at least 50 m from the nearest natural waterway.

## 6.2 Relevant biotic factors

70. The biotic factors pertaining to the growth and distribution of commercial canola are discussed in *The Biology of Brassica napus L. (canola)* (OGTR 2008). In addition, the following points are of particular relevance to this release:

- The proposed trial would take place in areas where canola and Indian mustard (*Brassica juncea*) are commercially grown.
- Under licence DIR 069/2006, the limited and controlled release of GM herbicide tolerant hybrid canola and Indian mustard would be permitted in the regions proposed for this release.
- Invertebrates, vertebrates and microorganisms are expected to be exposed to the introduced genes, their encoded proteins and end products.

## 6.3 Relevant agricultural practices

71. It is anticipated that the agronomic practices for the cultivation of the GM canola by the applicant will not differ significantly from industry best practices used in Australia. Plants at the release sites would therefore receive applications of water, fertilisers, herbicides, insecticides and other agronomic management practices similar to commercially grown canola plants. Conventional cultivation practices for canola are discussed in more detail in *The Biology of Brassica napus L. (canola)* (OGTR 2008).

72. 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 swathing or by direct harvesting. Swathing involves cutting the crop and placing it in rows to dry.

73. The applicant proposes to treat the GM canola with chemical desiccants pre-harvest to reduced pod shattering and to direct harvest, rather than swathe, the crop. Harvested material, including seed, would be transported to a contained facility for analysis. Any plant material remaining at the sites post harvest would be incorporated into the soil and allowed to decompose. Harvested areas would be managed to promote the germination of any residual seed through the use of fertilisers, irrigation and light tillage, and any volunteers would be destroyed. The sites would then be sown with a pulse, cereal or broad leaf crop, which will be monitored for volunteers.

## 6.4 Presence of related plants in the receiving environment

74. The proposed trial sites are at DPI Victoria research stations located in commercial canola growing regions. Commercial canola varieties grown in Victoria could include non-GM and GM varieties. GM glyphosate tolerant Roundup Ready® canola and GM glufosinate ammonium tolerant, hybrid InVigor® canola have been approved for commercial release under DIRs 020/2002 and 021/2002, respectively.

75. In addition, GM canola and Indian mustard lines containing genes for herbicide tolerance and/or a hybrid breeding system have been approved for limited and controlled release in the LGAs of Horsham and Southern Grampians (see DIR 069/2006). To date, GM canola and Indian mustard authorised under DIR 069/2006 have been planted at one site in Horsham, and have not been planted in Southern Grampians. The DIR 069/2006 Horsham site is approximately 20 km from the Horsham site proposed in the current application. Under licence DIR 069/2006, further plantings of GM canola and/or Indian mustard would be permitted in Horsham during the 2010 winter season and in the Southern Grampians during the summer season of 2010/2011. Details of the sites, if any, would be provided by the DIR 069/2006 licence holder prior to planting.

76. *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 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 2002a). 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 2002a). The applicant proposes to maintain a 50 m monitoring zone that is free of canola and any related species.

## **6.5 Presence of the introduced genes or similar genes and encoded proteins in the environment**

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

78. The introduced *ipt* gene was isolated from the common soil bacterium *A. tumefaciens*. Homologues of *ipt* and its encoded enzyme occur naturally in a wide range of higher plants, including plants widely consumed by animals and people, and in some microorganisms which are plant pathogens (Kamada-Nobusada & Sakakibara 2009).

79. The introduced *hph* gene is derived from the common gut bacterium *E. coli*, which is widespread in human and animal digestive systems as well as in the environment (Blattner et al. 1997). As such, it is expected humans routinely encounter the encoded protein through contact with plants and food.

80. Short regulatory sequences are derived from thale cress (*A. thaliana*), *A. tumefaciens* and CaMV. Although *A. tumefaciens* and CaMV are plant pathogens, the regulatory sequences comprise a small part of their total genome, and in themselves have no pathogenic properties.

## **Section 7 Australian and international approvals**

### **7.1 Australian approvals of GM canola**

#### **7.1.1 Previous releases approved by Genetic Manipulation Advisory Committee or the Regulator**

81. There has been no release of these GM canola lines in Australia. Dealings with the lines proposed for release have been undertaken in PC2 facilities under NLRDs 521/2002 and 522/2002.

82. The Regulator has issued two licences for the commercial release of GM canola containing genes for herbicide tolerance, either alone or combined with a hybrid breeding system (DIR 020/2002: Roundup Ready<sup>®</sup> canola and DIR 021/2002: InVigor<sup>®</sup> canola). These GM canola lines can now be grown in a number of States in Australia.

83. The Regulator has also issued six licences for the limited and controlled release of various GM canola lines. In addition, there have been field trials of GM canola lines under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC) (Table 3).

**Table 3. Summary of planned releases (PR) conducted under GMAC and DIR licences approved by the Gene Technology Regulator for GM *B. napus* in Australia**

Licence or Planned Release (PR) number	Title	Proponent	Trait
DIR 069/2006	Limited and controlled release of GM herbicide tolerant hybrid <i>Brassica napus</i> and hybrid <i>Brassica juncea</i>	Bayer CropScience Pty Ltd	Herbicide tolerance and hybrid breeding system
DIR 032/2002	Field Trial - Seed increase and field evaluation of herbicide tolerant hybrid canola	Bayer CropScience Pty Ltd	Herbicide tolerance and hybrid breeding system
DIR 021/2002	Commercial release of InVigor® hybrid canola ( <i>Brassica napus</i> ) for use in the Australian cropping system	Bayer CropScience Pty Ltd	Herbicide tolerance and hybrid breeding system
DIR 020/2002	General release of Roundup Ready® canola ( <i>Brassica napus</i> ) in Australia	Monsanto Australia Pty Ltd	Herbicide tolerance
DIR 011/2001	Field trials of Roundup Ready® canola ( <i>Brassica napus</i> ) in Australia in 2002	Monsanto Australia Pty Ltd	Herbicide tolerance
DIR 010/2001	Small and large scale trialing of InVigor® canola ( <i>Brassica napus</i> ) for development for the Australian cropping system	Aventis CropScience Pty Ltd	Herbicide tolerance and hybrid breeding system
PR-122	Development of canola cultivars with reduced yield loss	AgrEvo Pty Ltd	Reduced pod shattering
PR-121	Development of canola cultivars with modified plant architecture	AgrEvo Pty Ltd	Dwarf stature
PR-120	Development of methods to reduce anti-nutritional factors	AgrEvo Pty Ltd	Reduced glucosinolates
PR-111 and 132	Development of photoperiod insensitive canola cultivars ( <i>Brassica napus</i> )	AgrEvo Pty Ltd	Photoperiod insensitivity
PR-79, 93, 110, 119, and 133	Development of fungal disease resistant canola cultivars	AgrEvo Pty Ltd	Fungal resistance
PR-77	Planned release of transgenic canola expressing tolerance to the herbicide glyphosate (Roundup Ready® canola)	Monsanto Australia Pty Ltd	Herbicide tolerance
PR-63	Field evaluation of a genetically modified canola ( <i>Brassica napus</i> ) with a new hybridization system	AgrEvo Pty Ltd	Herbicide tolerance and hybrid breeding system
PR-62	Development of glufosinate ammonium tolerant canola cultivars	AgrEvo Pty Ltd	Herbicide tolerance
PR-60	Field evaluation of genetically modified canola ( <i>Brassica napus</i> ) for agronomic performance	Monsanto Australia Pty Ltd	Increased lauric acid
PR-14	Field evaluation of canola protoplast fusion breeding lines	Pacific Seeds Pty Ltd	Protoplast fusion

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

### **7.1.2 Approvals by other Australian government agencies**

85. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. Other government regulatory requirements may also have to be met in respect of release of GMOs, including those of the Australian Quarantine and Inspection Service (AQIS), Food Standards Australia New Zealand (FSANZ), and Australian Pesticides and Veterinary Medicines Authority (APVMA). This is discussed further in Chapter 3.

86. FSANZ is responsible for human food safety assessment and food labelling, including GM food. The applicant does not intend to use materials from the GM canola lines in human food, accordingly no application has been submitted to FSANZ. FSANZ approval would need to be obtained before materials from these GM canola lines could be sold as food or food ingredients.

### **7.2 International approvals of GM canola**

87. There have been no releases of these GM canola lines internationally. However, there have been numerous international releases of other GM canola lines. The traits that have been modified include herbicide tolerance, hybrid breeding system, increased yield, water use efficiency, nitrogen use efficiency, and modified oil composition<sup>8</sup>.

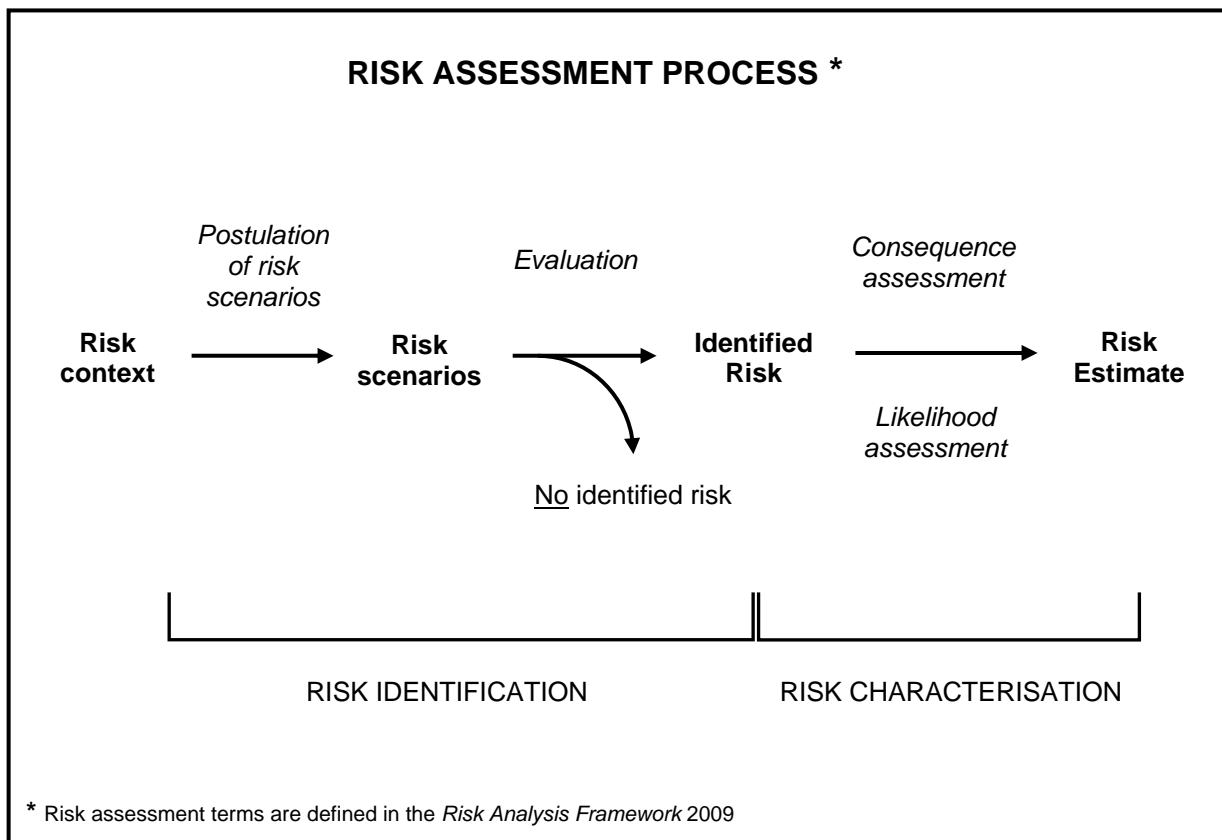
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<sup>8</sup> Sources: < <http://inspection.gc.ca/english/plaveg/bio/confine.shtml> >, <<http://www.aphis.usda.gov/brs/status/relday.html>>, <[http://gmoinfo.jrc.ec.europa.eu/gmp\\_browse.aspx](http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx)>, accessed 12 March 2010

## Chapter 2 Risk assessment

### Section 1 Introduction

88. 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.**

89. 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).

90. 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. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

91. 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 2009). In conjunction with these techniques, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

92. 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

93. 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
- the proposed controls
- 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.

94. Eight risk scenarios were identified and evaluated. These are summarised in Table 4, where circumstances that share a number of common features are grouped together in broader risk categories. None of the risk scenarios were considered to lead to an identified risk that required further assessment. More detail of the evaluation of these scenarios is provided later in this Section.

95. As discussed in Chapter 1, Section 5.2.4, the GM canola lines contain the antibiotic resistance selectable marker gene *hph*. The *hph* gene has already been considered in detail in the RARMP prepared for DIR 073/2007 and by other regulators (for example, EFSA 2004) and has been found to pose no risk either to people or the environment. Therefore, the potential effects of the *hph* gene will not be further assessed for this application.

**Table 4. Summary of risk scenarios from dealings with GM canola genetically modified for enhanced yield and delayed leaf senescence**

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms	1. Exposure to GM plant material containing the protein encoded by the introduced gene, or its end products	Allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>• The introduced gene, encoded protein and end products occur naturally in the environment and are unlikely to be toxic or allergenic to people or toxic to other organisms.</li> <li>• None of the GM canola material would be used for human food or animal feed.</li> <li>• The limited scale, short duration and other proposed limits and controls minimise exposure of people and other organisms to the GM plant material.</li> </ul>
Section 2.2 Spread and persistence (weediness) of the GM canola plants in the environment	2. Expression of the introduced gene improving the survival of the GM canola plants	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>• Many environmental factors are expected to limit the spread and persistence of canola in the areas proposed for release.</li> <li>• The limits and controls proposed for the release would minimise spread and persistence of the GM canola plants.</li> </ul>

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
	3. Dispersal of reproductive GM plant materials through various means, including animals and extreme weather conditions	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>Birds and animals are unlikely to disperse viable canola seed.</li> <li>Dispersal would be minimised by the proposed limits and controls, which include locating the sites away from natural waterways and transporting material according to the Regulator's guidelines.</li> </ul>
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	4. Expression of the introduced gene or regulatory sequences in other canola plants	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>The majority of pollen travels less than 10 m and the amount of pollen decreases with distance from the source.</li> <li>A 15 m pollen trap and 50 m monitoring zone will restrict pollen-mediated gene flow between the GM lines proposed for release and other canola plants.</li> <li>Locating the trial 400 m from any Brassica crop would further restrict gene flow to such crops.</li> <li>Risk scenarios 1 – 3 associated with expression of the introduced gene did not constitute identified risks for people or the environment.</li> </ul>
	5. Expression of the introduced gene in other sexually compatible plants	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>The majority of pollen travels less than 10 m and the amount of pollen decreases with distance from the source.</li> <li>The proposed limits and controls (eg the 15 m pollen trap and 50 m monitoring zone) would minimise gene flow.</li> <li>Risk scenario 1- 3 associated with expression of the introduced gene did not constitute identified risks for people or the environment.</li> </ul>
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	6. 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	<ul style="list-style-type: none"> <li>The introduced gene and regulatory sequences are already present in the environment and are available for transfer via demonstrated natural mechanisms.</li> <li>Risk scenarios 1 – 5 associated with expression of the introduced gene did not constitute identified risks for people or the environment.</li> </ul>
Section 2.5 Unintended changes in biochemistry, physiology or ecology	7. Changes to biochemistry, physiology or ecology of the GM canola plants resulting from expression, or random insertion, of the introduced genetic material	Weediness; allergic reactions in people or toxicity in people and other organisms	No	<ul style="list-style-type: none"> <li>Unintended, adverse effects, if any, would be minimised by the proposed limits and controls.</li> <li>Obvious unexpected alterations are likely to have been detected and eliminated during the production and laboratory screening of the GM canola lines.</li> </ul>

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.6 Unauthorised activities	8. Use of the GMOs outside the proposed licence conditions (non-compliance)	Potential adverse outcomes mentioned in Sections 2.1 to 2.5	No	<ul style="list-style-type: none"> <li>The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator.</li> </ul>

## 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms

96. 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).

97. 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).

98. A range of organisms may be exposed directly or indirectly to the protein (and end products) encoded by the introduced gene for enhanced yield and delayed leaf senescence. Workers cultivating the GM canola would be exposed to all plant parts. Organisms may be exposed directly to the protein 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). Indirect exposure would include organisms that feed on organisms that feed on GM canola plant parts or degrade them (vertebrates, invertebrates, fungi and/or bacteria).

### ***Risk scenario 1. Exposure to GM plant material containing the protein encoded by the introduced gene, or its end products***

99. Expression of the introduced gene for enhanced yield and delayed leaf senescence 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.

100. Non-GM *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).

101. 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  $\mu\text{moles g}^{-1}$  of glucosinolates in the meal. The levels of erucic acid and glucosinolates have not been measured in the GM canola plants. As cytokinins have been correlated with the accumulation of secondary metabolites in tobacco (Smigocki et al. 2000), it is possible that expression of IPT in the GM canola plants could affect the levels of erucic acid and glucosinolates. It is not known what, if any, effect expression of IPT may have on the levels of these metabolites. However, it is considered that any changes are unlikely to result in levels of erucic acid or glucosinolates outside the acceptable standards, as Australian canola varieties typically contain levels well below the current standards (<0.5 % erucic acid and < 20  $\mu\text{moles g}^{-1}$  glucosinolates, OGTR 2008).

102. Non-GM canola pollen, dust and flour have been implicated in allergic reactions in people and a number of putative allergens have been characterised (OGTR 2008). There is one report of cytokinins regulating a specific pollen allergen in soybean (Crowell 1994). However, cytokinins are

not generally associated with allergenicity of plants, and these properties are not expected to be altered in the GM canola lines proposed for release.

103. Although no toxicity or allergenicity studies have been performed on the GM canola plant material, the introduced gene was isolated from a common bacterium, which is already widespread and prevalent in the environment (see Chapter 1, Sections 5.2.3 and 6.5). The *ipt* gene is homologous to *ipt* genes present in higher plants. Therefore, people and animals are exposed to the same or similar proteins through their diet and the environment. No information was found to suggest that the *ipt* gene or its encoded protein are toxic or allergenic to people or other organisms. However, some uncertainty exists in this area due to data gaps.

104. Tobacco plants expressing the *ipt* gene have been found to adversely effect insects feeding upon them (see Chapter 1, Section 5.2.2). However, this effect was attributed to secondary metabolites of tobacco (Smigocki et al. 2000) and appears to be specific to *Nicotiana* species.

105. Canola is commonly utilised as a source of nectar and pollen for commercial honey production by honey bees. The percentage dry weight of canola pollen per wet weight of honey that is produced from hives placed in canola fields has been shown to be only 0.2% (Hornitzky & Ghalayini 2006). If the honey is sieved or filtered the pollen content is further reduced (discussed in Malone 2002). However, bee hives will not be introduced into the GM canola trial sites.

106. Most bees forage close to the hive and between neighbouring plants (Rieger et al. 1999), but long range foraging (up to 12 km) has been reported for honeybees under specific conditions (Beekman & Ratnieks 2000). Therefore, there is the potential for honeybees from commercial hives near the trial sites to visit the GM canola. Honeybees tend to visit only one plant species per trip, and will remain faithful to a particular species and foraging area as long as there is sufficient nectar or pollen available (see Scheffler et al. 1995 and references cited therein; Williams 2001).

107. The applicant proposes to surround the GM canola with a 50 m monitoring zone that is free of canola and related species, which would minimise the likelihood of bees foraging on the GM canola, as exclusion zones left unplanted or planted with a sexually non-compatible crop create an unattractive barrier to foraging bees (Williams 2001). In addition, the applicant proposes to surround the GM canola with a 15 m pollen trap of non-GM canola. Trap crops of only 6 m in width can restrict the transfer of GM pollen by bees by absorbing the GM pollen before it is transferred from the site (Williams 2001). Furthermore, the *ipt* gene is not expected to be expressed in the pollen from the GM canola due to the deletion of the pollen specific regions from the promoter used (see Section 5.3.1). Therefore, even in the unlikely event that some pollen was transported to a honey producing beehive, no exposure to the introduced protein would be expected.

108. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of exposure of people and other organisms to GM plant materials. The proposed trial sites are located at DPI Victoria research stations and will be surrounded by a fence with lockable gates, and only approved staff with appropriate training will have access to the site. This will reduce inadvertent access by humans and prevent grazing livestock from entering the site, which minimises exposure of the public and animals to the GM plant material. There is little potential for exposure of the public to GM plant material via ingestion, skin contact or inhalation as no GM plant material would be used for human food. Livestock and other animals would not be intentionally exposed as the GM plant material will not be used as feed.

109. Contact with, or inhalation of, GM plant materials would be limited to trained and authorised staff. Researchers and technical staff conducting the trials would be exposed to the GM plant materials during all phases of the trial. Workers may come into contact with the proteins encoded by the introduced gene when the plant cells have been damaged. Exposure to the GM canola is unlikely to lead to an adverse outcome as the GM canola plants are unlikely to be any more toxic or allergenic than non-GM canola.

110. After harvest, the applicant proposes to destroy GM canola materials produced, apart from retaining some plant materials for research purposes. These measures would minimise exposure to the GM plant material. The short duration (2010-2012) and small size (0.4 ha per year) of the proposed trial would also limit the potential for exposure to the GM plant material.

111. **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 proteins encoded by the introduced gene is **not** identified as a risk that warrants further assessment.

## **2.2 Spread and persistence (weediness) of the GM canola plants in the environment**

112. 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 2008). In summary, canola shares some characteristics with known weeds, ie having weedy relatives, high seed output, self- and cross-pollination and seed dormancy. Canola is considered a major weed in agricultural ecosystems in Australia and a minor weed in natural ecosystems (Groves et al. 2003). However, canola is a poor competitor and is not regarded as an environmentally hazardous colonising species. It is mainly a plant of disturbed habitats; unless the habitat is regularly disturbed, or seed replenished from outside, canola will be displaced by other plants (Salisbury 2002c).

113. The establishment, spread and persistence of canola populations is likely to be limited by water and nutrient availability, temperature and competition from other plants. To a lesser extent, high temperatures or frost during flowering as well as insect and disease pressures can greatly reduce seed set, thus limiting spread and persistence.

114. Scenarios that could lead to increased spread and persistence of the GM canola plants include expression of the introduced gene conferring tolerance to abiotic or biotic stresses, or increasing the dispersal potential of GM plant materials outside the release site. These risk scenarios could lead to increased exposure of vertebrates (including people), invertebrates and microorganisms to the encoded proteins or end products.

### **Risk scenario 2. Expression of the introduced gene improving the survival of the GM canola plants**

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

116. If the expression of the introduced gene for enhanced yield and delayed leaf senescence were to provide the GM canola plants with a significant selective advantage over commercially released GM or non-GM canola plants and if they were able to establish and persist in favourable non-agricultural environments, 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 agricultural environments if they exhibited a greater ability to establish and persist than commercially released GM or non-GM canola.

117. The impact of the genetic modification on survival of the GM canola plants is uncharacterised. However, a number of predictions can be made based on knowledge of the function of the *ipt* gene and on the observed phenotypes of other GM plants expressing the same gene (see Chapter 1, Section 5.2.2).

118. The GM canola lines proposed for release have been modified for enhanced yield and delayed leaf senescence. In the glasshouse, a range of leaf senescence and yield phenotypes have been observed. Delayed senescence and enhanced yield have also been observed in other GM plants expressing the *ipt* gene (Gan & Amasino 1995; Chen et al. 2001). In addition, expression of *ipt* has

resulted in various other effects in GM plants including morphological changes; altered seed development; increased resistance to the symptoms of bacterial infection; inhibition of fungal infection; enhanced insect resistance; enhanced water use efficiency; and tolerance to drought, waterlogging and freezing (see Chapter 1, Section 5.2.2). An increase in seed yield could impact on the spread and persistence of the GM canola plants. If the GM canola lines were to exhibit increased resistance to insects or diseases, or enhanced abiotic stress tolerance, their ability to spread and persist may be affected in environments where these factors were limiting.

119. The survival of the GM canola plants would still likely be limited by high temperatures, low intrinsic competitive ability, nutrient availability and other environmental factors that normally limit the spread and persistence of canola plants in Australia (OGTR 2008). Even if an increase in resistance or tolerance was conferred, the GM plants will most likely be less fit than other commercially available canola varieties because of the potential metabolic/physiological burdens associated with expression of the introduced *ipt* gene (Pretty 2001; Burdon & Thrall 2003; Denby & Gehring 2005). However, some uncertainty remains in this area due to data gaps.

120. The proposed release is for early stage research and the proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of the spread and persistence of the GM canola plants proposed for release. The release would be of limited size and short duration. The applicant proposes a number of control measures, including destruction of all plant materials not required for further analysis, post harvest irrigation and tillage to encourage germination of remaining seed and destruction of volunteers, and post harvest monitoring of the proposed sites for 24 months.

121. **Conclusion:** The potential for increased weediness, allergenicity or toxicity due to expression of the introduced gene for enhanced yield and delayed leaf senescence improving the survival of the GM canola plants is **not** identified as a risk that warrants further assessment.

**Risk scenario 3. Dispersal of reproductive GM plant materials through various means, including animals and extreme weather conditions**

122. If the GM canola plants were to be dispersed from the release sites they could increase the exposure of humans and other organisms to the GM plant material and/or establish and persist in the environment. The effects of contact, inhalation or ingestion of the GM canola plants have been assessed in Risk scenario 1 and were not an identified risk. The potential for the introduced *ipt* gene to result in improved survival of the GM canola plants in the environment was assessed in Risk scenario 2 and was not an identified risk.

123. Dispersal of reproductive GM plant materials, for example viable seed, could occur in a variety of ways including: endozoochory (dispersal through ingestion by animals), the activity of animals such as rodents and herbivores, or through extremes of weather such as flooding or high winds. Seed yield may be increased in the GM canola lines, but other dispersal characteristics are not expected to be altered compared to non-GM canola.

124. It is possible that canola seeds could germinate after passage through the digestive system of some mammals, but the proportion is likely to be very small. An Australian study found that canola seed was excreted from sheep for 5 days following dietary consumption (Stanton et al. 2003). Only 1-1.5% of canola seed ingested by sheep was excreted whole and the germination rate of this seed was 10-40% (Stanton et al. 2003). Therefore, less than 0.5% of ingested seed would be capable of germinating under field conditions and of this, only a small percentage would be expected to survive to maturity. In a German study of endozoochory, no intact rapeseed could be isolated from faeces of wild boar fed a mixture of maize and rapeseed (Wiedemann et al. 2009).

125. Similarly, it is unlikely that dissemination of GM canola seed by wild birds consuming seed directly from the crop would occur at a significant level. The potential for dispersal of canola seed by four dove/pigeon species, one finch species and two duck species has been examined using captive feeding trials (Twigg et al. 2008). While most of the birds tested ate at least some canola,

only one species of pigeon (crested pigeon) and one species of duck (wood duck) ate it in significant amounts in the presence of other food. Seed was generally well macerated after passage through the digestive tract. Out of 465 faecal pellets, whole seed was only found in seven, all of which were from wood ducks. A total of 11 whole seeds (<0.01% of seed ingested) were found, five of which successfully germinated. Omnivorous/herbivorous species such as ducks are less efficient at digesting seeds compared to most obligate seed-eaters. In contrast, parrots are even less likely to pass viable seed because they generally dehusk seeds and consume only the kernel (Twigg et al. 2008).

126. The canola seed is small and spherical and lacks dispersal characteristics such as stickiness, burrs and hooks, which can contribute to seed dispersal via animal fur (Howe & Smallwood 1982). It is possible that ants may remove seeds for underground storage but to depths where germination is highly unlikely. The proposed sites are at DPI Victoria research stations and are both surrounded by a stock-proof fence, limiting the possibility of seed dispersal by large animals or by unauthorised people accessing the site. Dispersal by authorised people entering the proposed trial site would be minimised by a standard condition of DIR licences which requires the cleaning of all equipment used at the trial site, including clothing.

127. Widespread natural dispersal of canola seeds does not generally occur in the field. Pod shattering can disperse seeds over very short distances. Extremes of weather, such as flooding or strong winds, may cause dispersal of plant parts. The applicant has stated that neither of the proposed trial sites is prone to flooding. The GM canola will be harvested directly (not swathed) with a dedicated harvester, limiting dispersal of the seed by wind. In addition, control measures have been proposed by the applicant to minimise dispersal outside the trial site (Chapter 1, Section 3.3). These include locating the proposed release sites away from natural water ways to prevent dispersal in the event of flooding, and transporting the GM plant materials in accordance with the Regulator's transportation guidelines.

128. **Conclusion:** The potential for allergenicity, toxicity or increased weediness due to the dispersal of reproductive GM plant materials through various means including animals and extreme weather conditions is **not** identified as a risk that warrants further assessment.

### 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants

129. Vertical gene flow is the transfer of genetic information 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 (Waines & Hegde 2003). For GM crops, vertical gene flow could therefore occur via successful cross-pollination between the crop and nearby canola plants, related weeds or native plants (Glover 2002).

130. 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.

131. Baseline information on vertical gene transfer associated with non-GM canola plants can be found in *The Biology of Brassica napus L. (canola)* (OGTR 2008). In summary, canola is predominantly self-pollinating with average inter-plant outcrossing rates of 30%. Most outcrossing between fields generally occurs within the first 10 m of the recipient field, and rates decline with distance.

***Risk scenario 4. Expression of the introduced gene or regulatory sequences in other canola plants***

132. Transfer and expression of the introduced gene for enhanced yield and delayed leaf senescence to other canola plants could increase the weediness potential, or alter the potential allergenicity and/or toxicity of the resulting plants.

133. All of the introduced regulatory sequences are expected to operate in the same manner as regulatory elements endogenous to the canola plants. While the transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects, the impacts from the introduced regulatory elements are likely to be equivalent to, and no greater than, those from endogenous regulatory elements.

134. As discussed in Risk scenario 1, allergenicity to people and toxicity to people and other organisms are not expected to be changed in the GM canola plants by the introduced gene. This will also be the case if the introduced gene is expressed in other GM or non-GM canola plants.

135. The survival of the GM canola plants proposed for release would be limited by a diverse range of environmental factors that normally limit the spread and persistence of canola plants in Australia (see Risk Scenario 2). This would also be the case if the introduced gene was transferred to other GM or non-GM canola plants and expressed in the progeny.

136. As a measure to restrict gene flow, the applicant proposes to surround the GM canola with a 15 m pollen trap planted with non-GM triazine tolerant canola. Outcrossing between the GM canola and the pollen trap plants is likely to occur. However, the triazine tolerance trait would only provide a selective advantage in situations where triazine is applied exclusively for weed control. The resulting hybrids could still be effectively controlled by mechanical means or the use of alternative herbicides. Plant material from the pollen trap plants would be destroyed, and the pollen trap area would be included in the post harvest monitoring proposed by the applicant.

137. As discussed in Chapter 1, Sections 6.4, licence DIR 069/2006 authorises the limited and controlled release of GM herbicide tolerant hybrid canola and/or Indian mustard in the regions proposed for this release. The release of these GM canola lines is required to be conducted under specific limits and controls that restrict gene flow. Two current licences also authorise the commercial release in Australia of GM canola lines containing genes for herbicide tolerance, either alone or combined with a hybrid breeding system (DIR 020/2002: Roundup Ready<sup>®</sup> canola and DIR 021/2002: InVigor<sup>®</sup> canola). These GM canola lines may also be grown in the regions proposed for this release. The applicant does not intend to use the commercial GM canola lines either as part of the trial or in the proposed pollen trap.

138. Canola is mainly self-pollinating, but outcrossing at levels of up to 55% between adjacent plants has been reported (Beckie et al. 2003). Canola pollen can be transferred by contact between plants, by wind or by insects. In general, wind borne pollen plays a minor role in long-distance pollination (McCartney & Lacey 1991). The vast majority of pollen travels less than 10 m and the amount of pollen decreases with distance from the source with a very steep decline over 50 m (Scheffler et al. 1993; Timmons et al. 1995). The dispersal range varies from a few metres to several hundred meters, but in extreme cases pollination over distances of up to 2 km has been attributed to wind (Timmons et al. 1995; Cai et al. 2008).

139. Insects, especially honeybees, play a more significant role in canola pollination over long distances (OECD 1997). Most bee movement occurs close to the hive and between neighbouring plants (Rieger et al. 1999), but foraging can occur regularly at distances of about 2 km from the hive (Ramsay et al. 1999) and, under specific conditions, foraging distances of up to 12 km have been reported (Beekman & Ratnieks 2000).

140. Canola pollen can remain viable for between 24 hours and one week (Mesquida & Renard 1982), with viability gradually decreasing over 4-5 days under natural conditions (Ranito-Lehtimäki

1995). Under very high temperatures, *Brassica* pollen can desiccate within a few hours (Salisbury 2006).

141. Outcrossing rates vary according to variety, local topography, environmental conditions, abundance of insect pollinators and experimental design, including the size and arrangement of donor and recipient populations. In general, smaller source plot sizes result in lower outcrossing rates (Timmons et al. 1995). Overseas field scale studies using male sterile or emasculated bait plants have detected outcrossing at rates of 5% at 4 km from the source plot (Thompson et al. 1999). Studies using male sterile or emasculated plants only represent the potential for gene flow and would greatly overestimate normal outcrossing levels due to lack of pollen competition (Salisbury 2002b).

142. Outcrossing rates are much lower when fertile recipient plants are used. A summary of studies in fully fertile *B. napus* concluded that most outcrossing occurs within the first 10 m of the recipient field and rates decline with distance (Husken & Dietz-Pfeilstetter 2007; Cai et al. 2008). Very low levels (<0.01%) of outcrossing have been detected at distances of up to 2 km in small scale studies (Cai et al. 2008), and up to 3 km from the source in field scale experiments (Rieger et al. 2002). However, the majority of small scale studies using fertile recipient plants report outcrossing distances of less than 400 m (Salisbury 2002a; Beckie et al. 2003; Husken & Dietz-Pfeilstetter 2007).

143. In general, levels of outcrossing beyond 400 m are irregular and presumably associated with bee activity (Salisbury 2002a). An exception to this is a recent Chinese study, which correlated both short- and long-distance pollen dispersal with wind direction and not bee numbers (Cai et al. 2008). Cai et al (2008) observed that while outcrossing levels decreased sharply with increasing distance from the source plot over short distances, dispersal and pollination were more random beyond 33.5 m (Cai et al. 2008).

144. The only published Australian study did not show any consistent decline in canola pollen dispersal over distance (Rieger et al. 2002). Instead, variable rates of outcrossing were observed, with isolated pollination events detected between 0 and 3000 m. Outcrossing occurred in 63% of the fields sampled but only a few had outcrossing rates greater than 0.03%. A rate of <0.01% was detected at 3 km. One main difference between the Rieger et al. (2002) study and the overseas studies was that the former utilized large 25- to 100-hectare pollen source fields, whereas the latter used relatively small pollen sources (<1 ha).

145. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would restrict the potential for pollen flow and gene transfer to commercially released GM or non-GM canola plants. In particular, the applicant proposes surrounding the trial sites with a 15 m pollen trap of non-GM canola and a 50 m monitoring zone that is free of canola, and locating the trial sites at least 400 m from any Brassica crop. The applicant also proposes to perform post harvest monitoring and to destroy any volunteer plants found at the sites.

146. **Conclusion:** The potential for allergenicity in people, toxicity in people and other organisms or increased weediness due to the expression of the introduced gene and regulatory sequences in other canola plants as a result of gene transfer is **not** identified as a risk that warrants further assessment.

#### **Risk scenario 5. Expression of the introduced gene in other sexually compatible plants**

147. Transfer and expression of the introduced gene for enhanced yield and delayed leaf senescence in other sexually compatible plants could increase the weediness potential, or alter the potential allergenicity and/or toxicity of the resulting plants.

148. As discussed in Risk scenario 1, allergenicity to people and toxicity to people and other organisms are not expected to be significantly changed in the GM canola plants by the introduced

gene. This will likely be the same if the introduced gene is expressed in other sexually compatible plants.

149. Expression of the introduced gene in other sexually compatible plants is unlikely to give these plants a significant selective advantage. The factors that limit the spread and persistence of hybrids between canola and other sexually compatible plants would be expected to limit the spread and persistence of any hybrids between the GM canola and other sexually compatible species.

150. As discussed in Chapter 1, Section 6.4, 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 2002a). Hybridisation would require synchronicity of flowering between the GM canola plants and sexually compatible species to enable cross-pollination and gene flow to occur.

151. Outcrossing from canola to cultivated vegetables of *B. napus* or *B. rapa* is possible if they are in physical proximity. Neither *B. napus* vegetables nor *B. rapa* vegetables are recognised as weeds in agricultural environments and they are generally harvested prior to flowering, unless they are grown for seed production, when precautions would usually be taken to maintain seed purity. Thus, hybrids between canola and vegetable crops of *B. napus* and *B. rapa* are unlikely to occur.

152. *B. oleracea* vegetables are cultivated in market garden regions in Australia. While there may be an occasional cultivation escape, *B. oleracea* is not naturalised as a weed in Australia (Salisbury 2002a). Hybrids between *B. oleracea* and *B. napus* are difficult to obtain artificially, and spontaneous hybrids from the wild have only been reported once at very low frequencies. These vegetable crops are also harvested prior to flowering.

153. *B. juncea* is grown commercially in Australia as a condiment mustard and a source of mustard oil. Like canola, Indian mustard is a weed in agricultural or disturbed areas (Groves et al. 2003), but it is not considered a significant weed of canola crops (Salisbury 2002a). Under natural conditions, outcrossing can occur between canola and Indian mustard, with recorded rates ranging from 3% to 4.7% when canola is the male parent and parent plants are in close proximity (Bing et al. 1991; Jorgensen et al. 1996). The reciprocal cross was less successful (Jorgensen et al. 1996; Jorgensen et al. 1998). *B. napus* x *B. juncea* hybrids have reduced pollen fertility (ranging from 0 to 28%) and low seed set, but have been shown to produce some viable seed and survive to the next generation (Bing et al. 1991; Jorgensen et al. 1996).

154. *B. rapa* has previously been grown in Australia as an oilseed crop but is no longer grown commercially in Australia. It is still present in the environment, primarily as a weed of agriculture and disturbed habitats. Naturally occurring hybrids between *B. napus* and *B. rapa* have been reported in both directions with spontaneous backcrossing and gene introgression occurring at low frequencies (Salisbury 2002a; Warwick et al. 2008). Studies of the fitness of *B. napus* x *B. rapa* hybrids have yielded varying results, but in general fertility is reduced and seed set is low (Salisbury 2002a; Warwick et al. 2008; Devos et al. 2009). In addition, hybrid seed lacks the dormancy of the persistent *B. rapa* parent (Linder 1998).

155. Naturally occurring hybrids between *B. napus* and sexually compatible species outside the tribe *Brassicaceae* have been reported at very low frequencies (approximately  $10^{-4}$  to  $10^{-8}$ ) for three important weed species in Australia; *R. raphanistrum*, *H. incana* and *S. arvensis* (Rieger et al. 2001; Darmency & Fleury 2000; Moyes et al. 2002; Salisbury 2002a).

156. Hybrids between non-GM herbicide tolerant *B. napus* and *R. raphanistrum* or *H. incana* were sterile or predominantly sterile with naturally occurring backcrossing from the hybrid to the non-canola parent occurring at low frequencies. In addition, after 5 generations of backcrossing, the herbicide tolerance trait had not been introgressed into the *R. raphanistrum* or *H. incana* genomes (Salisbury 2002a; Chevre et al. 2007).

157. When *R. raphanistrum* is the female the frequency of hybrid seed produced is lower than found on *B. napus* female plants (Salisbury 2002a). In an Australian study, no hybrids were detected among 25,000 seedlings grown from seed collected from *R. raphanistrum* plants that had been inter-planted in a field of non-GM herbicide tolerant canola (Rieger et al. 2001). Similarly, no interspecific hybrids were found in *R. raphanistrum* growing near herbicide tolerant *B. napus* fields in surveys done in the UK and Canada (Eastham & Sweet 2002; Warwick et al. 2003).

158. Naturally occurring hybrids between *S. arvensis* and *B. napus* have not been reported when both parents are fully fertile (Salisbury 2002a). Hybrids between *B. napus* and *S. arvensis* have been detected at extremely low frequencies with male sterile *B. napus* as the female recipient. Hybrids from crosses with *S. arvensis* as the female parent have only been reported when emasculation and hand-crossing are used (Moyes et al. 2002), and all hybrids are weak and sterile.

159. Natural hybrids between *B. napus* and other weed species in the tribe *Brassicaceae* have not been reported, although a few hybrids have been generated through controlled hand pollinations and embryo rescue. There have been no reports of hybrids, either naturally occurring or through controlled hand pollinations and embryo rescue, between *B. napus* and other weed species in tribes other than *Brassicaceae* (Salisbury 2002a).

160. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would restrict pollen flow and successful gene transfer to any sexually compatible plants. In particular, the applicant proposes to surround the site with a 15 m pollen trap and a 50 m monitoring zone that is free of related species, and to locate the sites at least 400 m from any Brassica crop.

161. **Conclusion:** The potential for allergenicity in people, toxicity in people and other organisms or increased weediness due to the expression of the introduced gene in other sexually compatible plant species as a result of gene transfer is **not** identified as a risk that warrants further assessment.

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

162. 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 occurs more frequently than previously believed and can occur 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.

163. Risks that might arise from horizontal gene transfer have been considered in previous RARMPs (for example see DIR 057/2004 and DIR 085/2008), 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 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.

164. Baseline information on the presence of the introduced or similar genetic elements is provided in Chapter 1, Section 6.5. All of the introduced genetic elements are derived from naturally occurring organisms that are already present in the wider Australian environment.

### **Risk scenario 6. Presence of the introduced genetic material in other organisms as a result of horizontal gene transfer**

165. Possible risks arising from HGT of the introduced genetic material to other organisms involves consideration of potential recipient organisms and the nature of the introduced genetic material. Risks that might arise through HGT from a GMO to another organism have been recently

reviewed (Keese 2008) and considered in detail in a previous RARMP (DIR 085/2008) which is available from the OGTR website <<http://www.ogtr.gov.au>> or by contacting the Office.

166. HGT could result in the presence of the introduced gene for enhanced yield and delayed leaf senescence in bacteria, plants, animals or other eucaryotes. However, the introduced gene was isolated from a bacterium that is already widespread in the environment (See Chapter 1, Section 6.5), and is thus already available for transfer from that source via demonstrated natural mechanisms.

167. 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 gene, the encoded protein or its end products are not associated with any risk then even in the unlikely event of HGT occurring, it should not pose any risk to humans, animals or the environment. Conclusions reached for Risk scenarios 1 - 5 associated with the expression of the introduced gene did not represent an identified risk. Therefore, any rare occurrence of HGT of introduced genetic material to other organisms is expected to be unlikely to persist and/or result in an adverse effect.

168. Baseline information on the presence of the introduced or similar genetic elements is provided in Chapter 1, Section 6.5. The introduced genetic elements are derived from organisms that are already present in the wider Australian environment.

169. **Conclusion:** The potential for an adverse outcome as a result of horizontal gene transfer is **not** identified as a risk that warrants further assessment.

## 2.5 Unintended changes in biochemistry, physiology or ecology

170. All methods of plant breeding can induce unanticipated changes in plants, including through pleiotropy<sup>9</sup> (Haslberger 2003). Gene technology has the potential to cause unintended effects due to the process used to insert new genetic material or by producing a gene product that affects multiple traits. Such pleiotropic effects may include:

- altered expression of an unrelated gene at the site of insertion
- altered expression of an unrelated gene distant to the site of insertion, for example, due to the protein encoded by the introduced gene changing chromatin structure, affecting methylation patterns or modulating/influencing signal transduction and transcription
- increased metabolic burden associated with high level expression of the introduced gene
- novel traits arising from interactions between the protein encoded by the introduced gene and endogenous non-target molecules
- secondary effects arising from altered substrate or product levels in the biochemical pathway incorporating the protein encoded by the introduced gene.

171. Such unintended pleiotropic effects might result in adverse outcomes such as toxicity or allergenicity; weediness, altered pest or disease burden; or reduced nutritional value as compared to the parent organism. However, accumulated experience with genetic modification of plants indicates that, as for conventional (non-GM) breeding programs, the process has little potential for unexpected outcomes that are not detected and eliminated during the early stage of selecting plants with new properties (Bradford et al. 2005).

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<sup>9</sup> Pleiotropy is the effect of one particular gene on other genes to produce apparently unrelated, multiple phenotypic traits (Kahl 2001).

***Risk scenario 7. Changes to biochemistry, physiology or ecology of the GM canola plants resulting from expression, or random insertion, of the introduced genetic material***

172. The applicant states that no secondary effects have been observed in the GM canola lines in the glasshouse, although they have undergone only limited characterisation as the project is in early stages. Considerations relevant to altered biochemistry, physiology and ecology, in relation to expression of the introduced gene, have already been discussed in Risk scenarios 1 to 3, and were not considered identified risks.

173. Various biochemical pathways of the GM canola plants could be changed by the expression of the introduced gene, resulting in the production of novel or higher levels of endogenous toxins, allergens or anti-nutritional compounds. Non-GM canola contains low levels of two natural toxicants, and canola pollen, dust and flour have been implicated in allergic reactions in people. For further discussion regarding the toxicity and allergenicity of non-GM canola see Risk scenario 1 and *The Biology of Brassica napus L. (canola)* (OGTR 2008).

174. The outcome of random insertion of an introduced gene is impossible to predict. Such outcomes may include, for example, alteration to reproductive capacity, altered capacity to deal with environmental stress, production of novel substances, and changes to levels of endogenous substances. Additionally, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003). During this limited and controlled release the applicant proposes to measure the agronomic performance of the GM canola lines, and any substantial unintended effects are likely to be detected during the trial.

175. The likelihood of any unintended effects causing adverse effects is minimised by the proposed limits and controls outlined in Chapter 1, Sections 3.2, and 3.3. In particular, the scale and duration of the trial would limit the potential for adverse effects. The proposed trial sites are at DPI Victoria research stations and both are surrounded by a fence, which limits exposure of the public and large animals to the GM plant material. Humans and livestock would not be intentionally exposed as the GM plant material will not be used as food or animal feed.

176. **Conclusion:** The potential for an adverse outcome as a result of altered biochemistry, physiology or ecology is **not** identified as a risk that warrants further assessment.

## **2.6 Unauthorised activities**

***Risk scenario 8. Use of the GMOs outside the proposed licence conditions (non-compliance)***

177. If a licence were to be issued, non-compliance with the proposed conditions of the licence could lead to spread and persistence of the GM canola plants outside of the proposed release areas and/or increased exposure of people and other organisms to GM material. The adverse outcomes that this risk scenario could cause are the same as those discussed in the sections above. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires that the Regulator has regard for the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities.

178. **Conclusion:** The potential for an adverse outcome as a result of unauthorised activities is **not** identified as a risk that warrants further assessment.

## **Section 3 Risk estimate process and assessment of significant risk**

179. The risk assessment begins with postulation of potential 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 non-GM parent organism and within the context of the receiving environment.

180. Eight risk scenarios were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether or not expression of the introduced gene could: result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if it occurred were also assessed.

181. 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.

182. The characterisation of the eight risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The principal reasons for this include:

- limits on the size, locations and duration of the release proposed by DPI Victoria
- suitability of controls proposed by DPI Victoria to restrict the spread and persistence of the GM canola plants and their genetic material
- limited ability and opportunity for the GM canola plants to transfer the introduced genes to commercial canola crops or other sexually related species
- none of the GM plant materials or products will be used in human food or animal feed
- 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.

183. In addition to the *ipt* gene assessed, the GM canola lines also contain the antibiotic resistance selectable marker gene *hph* (see Chapter 1, Section 5.2.4). The potential effects of the *hph* gene were not further assessed for this application as the *hph* gene has already been considered in detail in the RARMP prepared for DIR 073/2007 and by other regulators (for example, EFSA 2004) and has been found to pose no risk either to people or the environment.

184. Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM canola plants into the environment are considered to be **negligible**. Hence, the Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment.

## Section 4 Uncertainty

185. 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 (consequence and likelihood) are always uncertain to some degree.

186. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability<sup>10</sup>. For field trials, because they involve the conduct of research, some knowledge gaps are inevitable. This is one reason they are required to be conducted under specific limits and controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, rather than necessarily to treat an identified risk.

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<sup>10</sup> A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* (OGTR 2007) available at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>> or via Free call 1800 181 030.

187. For DIR 103, which involves early stage research, uncertainty is noted particularly in relation to the characterisation of:

- Risk scenario 1, regarding potential increases in toxicity or allergenicity as a result of the introduced gene
- Risk scenario 2, associated with a potential for increased survival of the GMOs
- Risk scenario 7, associated with the potential for any unintended effects as a result of changes in biochemistry, physiology or ecology of the GM canola plants.

188. Additional data, including information to address these uncertainties, would be required to assess possible future applications for a larger scale trial, reduced containment conditions, or the commercial release of these GM canola lines if they are selected for further development.

189. Chapter 3, Section 5 discusses information that may be required for future release.

## Chapter 3 Risk management plan

190. 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 is given effect through imposed licence conditions. The risk management plan informs the Regulator's decision-making process. In addition, the roles and responsibilities of other regulators under Australia's integrated regulatory framework for gene technology are explained.

### Section 1 Background

191. 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.

192. 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.

193. The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions imposed by the Regulator 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 Responsibilities of other Australian regulators

194. Australia's gene technology regulatory system operates as part of an integrated legislative framework. Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies<sup>11</sup>.

195. The *Gene Technology Act 2000* requires the Regulator to consult these agencies during the assessment of DIR licence applications. *The Gene Technology (Consequential Amendments) Act 2000* requires the agencies to consult the Regulator for the purpose of making certain decisions regarding their assessments of products that are, or contain a product from, a GMO.

196. FSANZ is responsible for human food safety assessment, including GM food. As the proposed trial involves early stage research, the applicant does not intend any material from the GM canola plants to be used for human food. Accordingly, the applicant has not applied to FSANZ to evaluate the GM canola plants. However, in the event of a commercial release, FSANZ approval would need to be obtained before materials from the GM canola plants could be sold for human consumption.

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<sup>11</sup> More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator. Free call 1800 181 030 or at <<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>>.

197. No other regulatory approvals are required.

### **Section 3 Risk treatment measures for identified risks**

198. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are **negligible** risks to people and the environment from the proposed trial of GM canola. The *Risk Analysis Framework* (OGTR 2009), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

199. These risk scenarios were considered in the context of the scale of the proposed release (a maximum area of 0.8 ha per growing season on two sites at Victorian government research stations, between May 2010 and May 2012), the proposed containment measures (Chapter 1, Section 3), and the receiving environment (Chapter 1, Section 6).

### **Section 4 General risk management**

200. Licence conditions have been imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment and limit the release to the size, locations and duration requested by the applicant. Both of these considerations were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and environment are negligible. The conditions are detailed in the licence and summarised in this Chapter.

#### **4.1 Licence conditions**

##### **4.1.1 Consideration of limits and controls proposed by DPI Victoria**

201. Sections 3.2 and 3.3 of Chapter 1 provide details of the limits and controls proposed by DPI Victoria in their application, these are discussed in the eight risk scenarios characterised for the proposed release in Chapter 2. The appropriateness of these controls is considered further below.

202. The proposed release will be confined to a maximum of 0.8 ha per growing season on two sites at Victorian government research stations in the LGAs of Horsham and Southern Grampians, and the duration of the proposed release will be limited to two years. None of the GM plant material is permitted to be used in human food or animal feed. Only staff with appropriate training will be allowed access to the trial sites. These measures will limit the potential exposure of humans, vertebrates and other organisms to the GMOs (Risk scenario 1) and the potential for the GM canola lines to disperse and establish outside the proposed release site (Risk scenario 3).

203. The applicant has stated that both trial sites are at least 500 m from the closest natural waterways and that neither site is prone to flooding, which reduces the likelihood of plant material being washed away from the site (Risk scenario 3). It is a standard DIR licence condition that a trial site be located at least 50 m from any natural waterway to limit the dispersal of viable GM plant material in the event of flooding.

204. As discussed in Chapter 1, Section 6.4 and Risk scenario 5, 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 2002a).

205. The applicant's proposal to restrict gene flow from the GM canola (Risk scenario 4 and Risk scenario 5) included locating the proposed trial sites at least 400 m from any *Brassica* crop and surrounding them with a 15 m pollen trap of non-GM canola plants and a 50 m monitoring zone. The applicant proposed to inspect for and destroy any related species occurring among the GM canola, in the pollen trap and in the monitoring zone during the flowering period of the GM canola.

206. Levels of outcrossing in canola decrease with increasing distance from the pollen source, with most outcrossing occurring within the first 10 meters of the recipient field. Very low levels of

outcrossing have been detected at distances of up to 3 km from the pollen source, but the majority of experimental studies report outcrossing at distances of less than 400 m (see Risk scenario 4).

207. Isolation distances required for international GM canola trials range from 50 – 400 m (Salisbury 2002a). For example, Canadian regulations require a 200 m isolation zone from *Brassica* species; or 10 m wide borders of synchronously flowering, non-GM *B. napus*; or growth of the GM canola plants in cages or bags. In addition, the GM canola plants must be isolated from weedy relatives by at least 50 m<sup>12</sup>. An isolation distance of 400 m is required for GM trials in France, Belgium and Sweden (Salisbury 2002a).

208. In Australia, requirements for basic and certified seed production for canola are aligned with Organisation for Economic Cooperation and Development (OECD) rules (Australian Seeds Authority Ltd. 2006). The production of basic seed requires an isolation of 200 m from other varieties or any other *Brassica* or cruciferous crop or weed species. The production of certified seed requires an isolation distance of only 100 m. OECD rules stipulate maximum acceptable levels of off-types or other cultivars of the same species of 0.1% for basic canola seed and 0.3% for certified canola seed (OECD 2008).

209. The applicant proposed a number of measures to restrict gene flow from the proposed trial sites. These include surrounding the GM canola with a 15 m pollen trap planted with non-GM canola. The use of pollen traps surrounding canola fields has been shown to be an effective measure to reduce pollen-mediated gene flow (Staniland et al. 2000). Studies have shown that, for short isolation distances, planting pollen trap plants is a more effective strategy for reducing pollen-mediated gene flow than leaving the area barren (Morris et al. 1994; Reboud 2003). A Canadian study examining the effectiveness of pollen traps of up to 30 m in width found that 95% of the outcross events occurred within the first 15 m of the pollen trap (Staniland et al. 2000). Therefore, the use of a 15 m pollen trap of synchronously flowering non-GM canola is expected to minimise gene transfer to sexually compatible plants and is imposed as a licence condition.

210. The applicant will surround each pollen trap with a 50 m monitoring zone within which inspections for canola and related species are required during the flowering period of the GMOs. If found, these must either be destroyed or prevented from flowering. The applicant can apply to the Regulator for permission to grow an unrelated plant species in this area to assist with weed control and soil stability. The 50 m monitoring zone is expected to reduce bee-mediated pollen transfer. As discussed in Chapter 2, Risk Scenario 1, honeybees tend to visit only one plant species per foraging trip (see Scheffler et al. 1995 and references cited therein), and a zone of unrelated plants creates an unattractive barrier to bees (Williams 2001). While unrelated plants grown in the monitoring zone may be visited by bees, it is unlikely that any bees foraging on these plants would also forage on the GM canola, other canola or other related species.

211. The applicant also proposed to maintain a 400 m separation between the GM canola lines and any other *Brassica* crop, such that the isolation zone will extend 400 m from the edge of the GM canola plants and 385 m from the edge of the pollen trap. On the basis of the scientific literature on gene flow, international containment measures for GM canola trials, and the rules for producing basic and certified seed, preventing the planting of *Brassica* crops within 400 m of the GM canola lines is considered adequate to minimise gene flow from the GM canola to any *Brassica* crops being grown for breeding, commercial or research purposes.

212. Hybridisation between the GM canola and other sexually compatible species would be possible if they were in close proximity and there was synchrony of flowering. Hybrids between *B. napus* and *B. rapa* or *B. juncea* generally have reduced fertility and low seed set, with gene introgression

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<sup>12</sup> Source: <http://www.inspection.gc.ca/english/plaveg/bio/dt/term/2009/branape.shtml>, accessed 7 April 2010

occurring only at low frequencies (Salisbury 2002a). Hybrids between *B. napus* and *B. oleracea* are difficult to obtain (Ford et al. 2006). Similarly, hybrids between *B. napus* and *R. raphanistrum*, *H. incana* or *S. arvensis* are rare and are generally sterile or predominantly sterile (Salisbury 2002a).

213. The vast majority of pollen travels less than 10 m and the amount of pollen decreases with distance from the source (Scheffler et al. 1993; Timmons et al. 1995). It is expected that the 15 m pollen trap will absorb most of the pollen available for transfer from the GM canola plants and provide a source of non-GM pollen. The 50 m monitoring zone kept free of canola and related weedy species while the GMOs are flowering will further restrict gene flow to sexually compatible species.

214. In determining post-harvest monitoring requirements, one important consideration is the potential dispersal of seed during sowing and harvesting (mechanical dispersal). This is most likely to result in dispersal of seed into the area immediately around the trial, including the monitoring zone.

215. Canola can produce large numbers of small seeds (average seed weight of 5 mg) which can result in significant losses during sowing, harvest and transportation as well as losses from plants in the field due to pod shattering. Average losses of 3000 viable seeds/m<sup>2</sup> have been reported in Canada (Gulden et al. 2003), and losses of up to 10,000 seeds/m<sup>2</sup> have been measured in the United Kingdom (Lutman 1993). This can result in high densities of these plants occurring as weeds ('volunteers') in subsequent crops (Legere et al. 2001). However, seed losses vary greatly from year to year and also with crop life cycle and harvest method (Harker et al. 2006). Seed losses in spring sown canola have been measured at less than half that of winter sown canola (Price et al. 1996).

216. The survival of canola seed in the seedbank is very low compared with wild relatives (Chadoeuf et al. 1998). Canola has no primary dormancy and most volunteers germinate within 1 - 2 years following harvest (Salisbury 2002a; Harker et al. 2006; Mewett et al. 2008). However, induced secondary dormancy can result in some canola seeds surviving in the soil for several years, with European studies reporting dormancy of up to 11 years (Gruber et al. 2008). Induction of secondary dormancy depends on genotype, environmental conditions during seed maturation and post harvest conditions (Gruber et al. 2005). Post harvest conditions that can induce secondary dormancy in canola seeds include long exposure to darkness and low water availability (Gulden et al. 2004; Gruber et al. 2008).

217. Studies in Canada and Europe have shown that incorporation of seeds into the soil immediately after harvest (usually via tillage) exposes seeds to dormancy inducing conditions (i.e. dry soil and darkness) and usually results in a large soil seed bank (Gruber et al. 2005; Harker et al. 2006; Gruber et al. 2008; Gruber et al. 2010). Therefore, delaying tillage for 2 – 4 weeks can result in smaller seedbanks and reduced secondary dormancy and persistence (Harker et al. 2006; Gruber et al. 2008). Models have confirmed these field observations and suggest delaying tillage as long as possible or at least until it has rained enough to moisten the top soil layers and allow seeds to imbibe sufficient water for germination (Colbach et al. 2008). In the case of winter grown canola in Australia, leaving residual seed on the surface for a period of time could also expose it to very high soil surface temperatures that can contribute to seed death (Gulden et al. 2004). Therefore, retention of dropped seed on the soil surface for at least 28 days is imposed as a licence condition.

218. As well as timing of tillage, the method and depth of cultivation can also affect seed persistence and volunteer emergence (Gruber et al. 2008). In general, seeds are more likely to persist at deep rather than shallow depths (Andersson & Vicente 2010). Greenhouse experiments have shown that the majority of canola seeds emerge from depths of 1 – 5 cm. Germination and emergence were reduced at 0 and 7 cm and completely inhibited at 12 cm (Gruber et al. 2010). In field studies in Europe, shallow non-inversion tillage, where the seeds remain in the upper 10 cm of the soil, results in increased emergence of volunteers within the first year following harvest. In contrast, ploughing to a depth of 10 – 20 cm preserves seeds in deep soil layers (Gruber et al. 2005;

Gruber et al. 2008). Shallow tillage under suitable conditions will also serve to enable degradation of the plant material remaining at the sites after harvest and is imposed as a licence condition.

219. Very few studies of canola seed persistence have been conducted under Australian conditions. In one study of farmer-managed fields in South Australia, soil samples were collected from minimum- and no-tillage farms known to have had a canola crop in the past three years (Baker & Preston 2008). Samples were collected at 0.5, 1.5, 2.5 or 3.5 years after harvest and were used to estimate the number and viability of canola seed over time. The variety of canola that had been grown was not considered as a factor in this study. At 2.5 years post harvest, 50-140 seeds/m<sup>2</sup> were recovered from the surveyed fields, but an average of less than 2% germinated. By 3.5 years post harvest, none of the recovered seeds germinated. Overall, the data indicate that the number of seeds recovered and the proportion that germinated declined rapidly with time (Baker & Preston 2008).

220. Monitoring requirements have been completed for several GM canola trials in Australia, and the data collected provides further information about canola seed persistence in Australian conditions. The applicant proposes to plant the GM canola lines as both a winter and summer crop. Analysis of the monitoring reports indicates that the germination pattern of volunteers varies for winter grown canola compared to summer grown canola. For winter sown GM canola, the vast majority of volunteers germinate in the first year, with relatively few volunteers in the second year, and none in year three for the majority of trials (Salisbury 2002a). For summer grown GM canola, the germination pattern of volunteers is more variable and delayed germination is more common. In approximately half of the summer grown trials, most volunteers germinated in the first year. In the other half of trials, the majority of germination occurred in years 2 and/or 3, and for some trials germination of volunteers was also reported in year four. One trial site was volunteer-free for a period nine months before further volunteers emerged in the third year after harvest.

221. The applicant proposed a number of measures to minimise the persistence of any GM canola plants and seeds in the seed bank at the proposed release site after harvest of the proposed trial (Risk scenario 2). These measures include treating harvested areas to encourage germination of any residual seed with light tillage and applications of water, fertilisers and herbicides similar to normal agricultural practices. Plant material remaining at the site will be incorporated into the soil at an unspecified time after harvest and allowed to decompose. The applicant also proposed to monitor the proposed release sites for 24 months after harvest on a monthly basis and to destroy any volunteers by hand removal or herbicide application. In addition to this, it is considered that post harvest monitoring of the release sites should continue until no volunteers are detected for at least 12 continuous months.

222. The combination of these measures would effectively reduce survival and persistence of viable canola seeds in the soil. Therefore, the licence conditions require: post harvest monitoring for at least 24 months, and until no volunteers are observed in the most recent 12 month period; and at least two light tillage events, the first occurring between 28 and 60 days after harvest and another occurring in the 12 month volunteer-free period prior to an application to sign-off a site. Tillage must not bury plant material to a depth of more than 5 cm and must occur in conditions where germination of the GMOs is reasonably likely to ensue (eg after irrigation or rainfall). These treatments will promote germination by ensuring any remaining seeds are placed at an appropriate depth in conditions that promote germination and will also encourage the microbial decomposition of any residual seed. These measures will minimise the persistence of the GMOs in the environment (Risk scenario 2).

223. The applicant proposed to grow other crops at the sites following harvest and cleaning. The OGTR's Policy on Post Harvest Crops (available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/policies-1>) lists a number of crop species that have been assessed in relation to their effect on the detection and control volunteer GM *Brassica* plants and that are permitted on GM *Brassica* field sites in the post harvest monitoring period. The post harvest crops proposed by the applicant include some that are listed in the Policy

on Post Harvest Crops, such as cereals (wheat, barley or oats), pulses (chickpeas or lentils) or pasture species. In addition, the applicant proposed planting field peas as a post harvest crop. Field peas are not currently listed in the OGTR's Policy on Post Harvest Crops. Field peas (*Pisum sativum*) are legumes that range in growth habit from trailing/climbing to erect at maturity. Plant height depends on temperature and light as well as variety, but generally field peas grow 30 - 150 cm in height. Although field peas can be distinguished from canola plants based on leaf morphology and flower colour, it is considered likely that the presence of field peas would impede the detection and timely control of volunteer canola plants. Therefore, field peas are not permitted as a post harvest crop.

#### **4.1.2 Summary of measures imposed by the Regulator to be implemented to limit and control the release**

224. A number of licence conditions have been imposed to limit and control the release, based on the above considerations. These include requirements to:

- limit the release to a total area of up to 0.8 ha per growing season at two sites at Victorian Government research stations within the LGAs of Horsham and Southern Grampians, between the date of issue of the licence and May 2012
- locate the trial sites at least 50 m away from natural waterways
- surround the GM canola with a 15 m pollen trap of non-GM canola plants and a 50 m monitoring zone in which sexually compatible species are not permitted to flower
- ensure no other *Brassica* crops are grown within 400 m of the GM canola plants
- harvest the GM canola plant material separately from other crops
- clean the sites and equipment used on the sites following harvest
- apply measures to promote germination of any canola seeds that may be present in the soil after harvest, including shallow tillage
- monitor the site for at least 24 months after harvest and destroy any canola plants that may grow until no volunteers are detected for a continuous 12 month period
- destroy all GM plant material not required for further analysis or future planting
- transport material from the GMOs in accordance with Regulator's guidelines
- not permit any GM canola plant material to be used in human food or animal feed.

## **4.2 Other risk management considerations**

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

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

### **4.2.1 Applicant suitability**

226. 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 applicant's history of compliance with previous approved dealings
- the capacity of the applicant to meet the conditions of the licence.

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

228. 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.

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

#### **4.2.2 Contingency plan**

230. DPI Victoria is required to submit a contingency plan to the Regulator within 30 days of the issue date of the licence. This plan must detail measures to be undertaken in the event of any unintended presence of the GM canola lines outside of the permitted areas.

231. DPI Victoria is also 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 is required within 30 days of the issue date of the licence.

#### **4.2.3 Identification of the persons or classes of persons covered by the licence**

232. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence.

#### **4.2.4 Reporting requirements**

233. 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 trial
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the trial.

234. The licence holder is also obliged to submit an Annual Report within 90 days of the anniversary of the licence containing any information required by the licence, including the results of inspection activities.

235. A number of written notices are also required under the licence that would assist the Regulator in designing and implementing a monitoring program for all licensed dealings. The notices include:

- expected and actual dates of planting
- expected and actual dates of flowering period
- expected and actual dates of harvest and cleaning after harvest.

#### **4.2.5 Monitoring for Compliance**

236. 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. Post-release monitoring

continues until the Regulator is satisfied that all the GMOs resulting from the authorised dealings have been removed from the release sites.

237. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

238. 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 5 Issues to be addressed for future releases***

239. Additional information has been identified that may be required to assess an application for a large scale or commercial release of these GM canola lines, or to justify a reduction in containment conditions. This includes:

- additional data on the potential toxicity and allergenicity of plant materials from the GM canola lines
- additional phenotypic characterisation of the GM canola lines, in particular of traits which may contribute to weediness, including abiotic and biotic stress tolerance
- characterisation of the introduced genetic material in the plants, including copy number and expression pattern.

### ***Section 6 Conclusions of the RARMP***

240. The risk assessment concluded that this proposed limited and controlled release of up to 10 GM canola lines on a maximum total area of 0.8 ha per growing season over two years at two Victorian government research stations in the LGAs of Horsham and Southern Grampians, poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

241. The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the release to the size, locations and duration proposed by the applicant, as these were important considerations in establishing the context for assessing the risks.

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## Appendix A Summary of issues raised in submissions received from prescribed experts, agencies and authorities<sup>13</sup> on the consultation RARMP for DIR 103

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

Summary of issues raised	Comments
The bacterial form of the derived <i>isopenentyltransferase</i> gene is only 40- 60% similar to the plant derived gene which raises a question of allergenicity. Will this be considered in future testing?	The RARMP for this limited and controlled release outlines future research requirements should the applicant seek a licence for a larger scale or commercial release. The research requirements include further information on the potential allergenicity or toxicity of the GM plants (see Ch 3, Section 5).
All risks seem to be dismissed too quickly with only vague assessment lacking any quantitative or qualitative measures. More rigor in the guidelines would be a benefit. Concerned that the RARMP dismisses possible areas of risk of harm because risk of harm is managed by only permitting a limited and controlled release. OGTR should concede there is a risk of harm (due to the unknown nature of the modification) and this risk of harm will be minimised by only permitting a limited and controlled release.	RARMPs are prepared using the risk analysis model and terminology as described in the Regulator's <i>Risk Analysis Framework</i> (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, which includes the limits and controls proposed by the applicant. 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. Eight risk scenarios were postulated in the RARMP prepared for this release. The characterisation of these risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not identify any risks that required further assessment.
There should be no claims made without proof being quoted. For example, paragraph 102 states any changes to concentrations of erucic acid and glucosinolates are unlikely to lead to the canola containing greater than the Australian standards. As IPT is known to lead to a range of outcomes, it seems a bit premature to use the work unlikely – unknown might be better. Paragraphs 103 and 104 conclude that the GM canola plants are unlikely to be more toxic or allergenic but there is uncertainty. Suggests it would be better to state that as it is not known whether the GM canola plants will be more allergenic, the risk of harm is minimised by restricting the release to a limited and controlled release.	Ch 2, Risk Scenario 1 has been modified to include a sentence stating that it is not known what effect, if any, expression of IPT may have on the levels of erucic acid and glucosinolates in the GM canola plants. The proposed release involves early stage research, and the RARMP notes that uncertainty exists, including in relation to potential increases in the toxicity or allergenicity of the GM canola plants as a result of the introduced gene. Additional data to address these uncertainties would be required to assess future applications for a larger scale trial, reduced containment conditions, or the commercial release of these GM canola lines if they are selected for further development. The potential for allergic reactions in people, or toxicity in people and other organisms, as a result of exposure to the GM canola lines was assessed in the context of the limits and control measures proposed by the applicant and was not identified as a risk that warranted further assessment.

<sup>13</sup> GTTAC, State and Territory Governments, Australian Government agencies and the Minister for the Environment.

Summary of issues raised	Comments
The risk of people doing the wrong thing is not taken into account.	The potential for unauthorised activities was considered in Risk Scenario 8 and the RARMP concluded that there were negligible risks to people or the environment from this release of GM canola. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs.
Section 2.5, 171, page 30: Plant breeding and GM breeding are not similar and do not carry the same risks.	Ch 2, Section 2.5 refers to the potential for unintended effects in the GM plants. Risk Scenario 7 evaluates the potential for harm as a result of unintended effects and this scenario was not identified as a risk that warranted further detailed assessment. All methods used for plant breeding, including conventional (non-GM) breeding, can induce unanticipated changes in plants. For plants generated by gene technology, unintended effects may also arise from the process used to insert new genetic material or by producing a gene product that affects multiple traits. The likelihood of any unintended effects causing adverse effects is minimised by the proposed limits and controls outlined in Chapter 1, Sections 3.2, and 3.3. In particular, the scale and duration of the trial would limit the potential for adverse effects. It is a condition of all DIR licences that any unexpected or unintended effects of the GMO be reported to the Regulator.
Suggests that language more in line with reality such as “minimal risk” could be used.	RARMPs are prepared using the risk analysis model and terminology as described in the Regulator’s <i>Risk Analysis Framework</i> (RAF), which is based on the internationally recognised Australia-New Zealand Standard on Risk Management (AS/NZS 4360:2004). Risk assessment terms used are defined in the RAF, and some of these definitions are included in the RARMP where relevant.
This risk assessment is only appropriate for trial licences not for general release.	Agreed. Each application for a DIR licence is assessed on a case by case basis. The applicant proposed an experimental release of small size and limited duration and with controls, and the Regulator assessed it within this context. An application for a larger scale or commercial release of these GM canola lines would require the preparation of a new RARMP by the Regulator.
If conventionally bred canola is grown at the same research stations as the GM canola, the unintended spread of GM canola is more likely than when the GM plants are grown in an area far (several km) from a breeding station. Cross contamination via pollination or through shared equipment is reduced by the suggested measures in DIR103 (but they only reduce the risk of spread and persistence of GM plants). In the rare case that contamination between GM canola and a conventional breeding line occurs, it is more likely that the GM event is maintained as the breeding lines are used for further crosses in the breeding process. If contamination occurs in a field far from the research farm, the harvested crop would be processed and not used for intentional crosses. Suggests that sites other than the breeding stations are used, unless it can be shown that there is no conventionally bred canola grown at the same time.	On the basis of the scientific literature on gene flow, international containment measures for GM canola trials, and the rules for producing basic and certified seed, a 400 m isolation zone clear of any other <i>Brassica</i> crop is considered adequate to restrict gene flow from the GM canola plants to other canola crops being grown for breeding, commercial or research purposes.

Summary of issues raised	Comments
<p>Notes that expression of the <i>ipt</i> gene may improve plant resistance to insects and diseases (references provided). This could increase the weediness of GM canola, especially in the event of stacking with other traits (eg waterlogging and drought tolerance). Therefore, recommends that relevant data regarding any potential selective advantage conferred by expressing the <i>ipt</i> gene should be collected during this trial to improve the confidence in assessing the environmental safety of any future commercial release of these GM canola lines.</p>	<p>Ch 1 Section 5.2.2 of the RARMP has been modified to incorporate the references provided in which expression of the <i>ipt</i> gene is shown to improve plant resistance to insects and diseases. In addition, Risk Scenario 2 has been modified to include increased resistance to insects or diseases in the description of potential phenotypic effects of expression of the <i>ipt</i> gene in the GM canola plants.</p> <p>The RARMP for this limited and controlled release outlines future research requirements should the applicant seek a licence for a larger scale or commercial release. The research requirements include additional phenotypic characterisation of the GM canola lines, in particular of traits which may contribute to weediness, including abiotic and biotic stress tolerance and this has been highlighted to the applicant.</p>
<p>Advise that the wording of the containment measures preventing pollen flow be clarified.</p>	<p>Ch 3, Sections 4.1.1 and 4.1.2, have been modified to reflect that licence conditions prohibit flowering of canola or other related species in the 50 m monitoring zone around each trial site.</p>
<p>Advise that data on the levels of erucic acid and glucosinolates in the GM canola might be required in support of a future application for commercial release.</p>	<p>The RARMP outlines future research requirements should the applicant seek a licence for a larger scale or commercial release. The research requirements include further information on the potential allergenicity or toxicity of the GM plants, such as erucic acid and glucosinolate levels.</p>

## Appendix B Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 103

The Regulator received one submission from the public on the consultation RARMP. This submission, 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.

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

**Issues raised:** C: Controls; GT: Gene Transfer; H: Human health

**Other abbreviations:** Ch: Chapter; GM: Genetically Modified; GMO: Genetically Modified Organism; LC: Licence Conditions; RARMP: Risk Assessment and Risk Management Plan.

**Type:** I: individual; G: Group.

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
1	I	x	H	Believes that GMOs are a threat to the food chain and to human health.	<p>DIR 103 is a limited and controlled release for the purpose of conducting experiments, including field characterisation of the GM canola lines.</p> <p>The Regulator has imposed a range of measures to minimise exposure to the GMOs and their genetic material including preventing use in food and feed and to limit the trial to the proposed size, locations and duration.</p> <p>The RARMP concluded that this limited and controlled release poses negligible risks to people or the environment.</p> <p>Furthermore, the RARMP outlines future research requirements should the applicant seek a licence for a larger scale or commercial release. The research requirements include further information on the potential allergenicity or toxicity of the GM plants.</p>
			C, GT	Grows traditional, open pollinated, heritage vegetable and fruit varieties. These efforts would be directly threatened by contamination from GMOs.	<p>The trial is of small size and limited duration, and licence conditions have been imposed to restrict the spread and persistence of the GMOs and their genetic material in the environment. These include a requirement to surround the GM canola with a 15 m pollen trap and to maintain a 400 m isolation zone around each trial site in which no sexually compatible species, including any other <i>Brassica</i> crop, can be grown.</p>