Australian Government
Department of Health and Ageing
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for **DIR 069/2006**

Limited and controlled release of GM herbicide tolerant hybrid *Brassica napus* and *Brassica juncea*

Applicant: Bayer CropScience Pty Ltd

March 2007

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Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence for dealings involving the intentional release of genetically modified (GM) canola and Indian mustard lines containing genes for herbicide tolerance and a novel hybrid breeding system into the environment, in respect of application DIR 069/2006 from Bayer CropScience Pty Ltd (Bayer).

The DIR 069/2006 licence permits the release of the GM canola and Indian mustard lines on a limited scale and under controlled conditions.

The *Gene Technology Act 2000* (the Act) and the *Gene Technology Regulations 2001* (the Regulations) and corresponding State and Territory law govern the process undertaken by the Regulator before a decision is made on whether or not to issue a licence. The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with the *Risk Analysis Framework* and in consultation with a wide range of experts, agencies and authorities, and the public.

More information on the comprehensive assessment undertaken for 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/>.

The application

Bayer applied for a licence to release GM canola and Indian mustard lines containing introduced genes for herbicide tolerance and a hybrid breeding system into the environment, on a limited scale and under controlled conditions. The trial will take place on a maximum of 252 hectares, comprising up to 42 sites of no more than 6 ha per site, over 6 seasons between 2007 and 2010. Up to 8 sites per winter and 6 sites per summer season will be used. Potential sites have been identified in 24 shires in New South Wales, South Australia and Victoria.

Bayer's novel breeding system emulates the natural phenomenon of hybrid vigour when progeny of genetically distinct parents provide improved agronomic performance over the parental lines. The GM canola and Indian mustard lines contain either the *barnase* gene or *barstar* gene which confer male sterility (MS) or restore fertility (Rf), respectively. Conventional breeding between genetically modified MS and Rf lines results in GM hybrid lines with restored fertility. Bayer may release genetically modified MS, Rf and hybrid lines of canola and Indian mustard. All GM canola and Indian mustard lines are also herbicide tolerant.

In accordance with the provisions of section 185 of the *Gene Technology Act 2000*, Bayer sought and received approval for certain information, including details of the trait for herbicide tolerance, to be declared Confidential Commercial Information (CCI). The CCI was made available to the various prescribed experts and agencies that are required to be consulted on the preparation of all RARMPs for DIR applications.

The purpose of the trial is to evaluate agronomic traits such as herbicide tolerance, germination efficiency, and flowering times in the GM canola and Indian mustard lines compared to conventional non-GM canola and Indian mustard, as well as previously approved GM InVigor® canola lines.

Seed of the GM canola and Indian mustard lines will be imported from Canada. Seeds collected during the trial may be shipped back to Canada for further trait evaluation. Bayer envisages that seeds from promising lines may be further assessed in future seasons in Australia (subject to further approvals).

Bayer proposed a number of measures to limit the spread and persistence of the GMO and the introduced genetic materials that were considered during the evaluation of the application. None of the GM plants materials, or their by-products, will be used for stock feed or human food.

Risk assessment

The hazard identification process considered the circumstances by which people or the environment may be exposed to the GMOs, GM plant materials, GM plant by-products, the introduced genes, or products of the introduced genes.

A hazard (source of potential harm) may be an event, substance or organism. A risk is identified when a hazard is considered to have some chance of causing harm. Those events that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

Nineteen events were identified and assessed whereby the proposed release of the GM canola and Indian mustard lines might give rise to harm to people or the environment.

These 19 events included consideration of whether 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 plants, or produce unintended changes in their biochemistry or physiology. In addition, consideration was given to the opportunity for gene flow to other organisms, and its effects if this occurred.

All events were characterised in relation to both the magnitude and probability of harm in the context of the controls proposed by the applicant to limit the spread and persistence of the GMOs in both time and space. This detailed consideration concluded that none of the nineteen events gave rise to an identified risk that required further assessment. The principal reasons comprise:

- the scale of the trial is limited in both area and duration
- containment, monitoring and disposal measures proposed by the applicant to limit the spread and persistence of GM canola and Indian mustard plants
- none of the GM plant materials or products from the GM plants 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 allergenicity from these proteins
- limited capacity of the GM canola and Indian mustard lines to spread and persist in natural ecosystems or undisturbed areas
- limited ability and opportunity for the GM canola and Indian mustard lines to transfer the introduced genes to related species or other organisms.

Therefore, any risk of harm to the health and safety of people, or the environment, from the limited and controlled release of the GM canola and Indian mustard lines is considered to be **negligible**.

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment. As none of the 19 events characterised in the risk assessment are considered to give rise to an identified risk that requires further assessment, the level of risk is considered to be **negligible**.

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation. However, conditions have been imposed on the licence to restrict the release to the size, duration and locations requested by the applicant, as these were an important part of establishing the context for assessing the risks.

The licence conditions require the applicant to limit the size and duration of the release to a maximum total area of 252 hectares over 3 years (2007-10) and prevent the use of the GMOs, or materials from the GMOs for any other purposes. Containment measures include maintaining physical isolation of the release sites; transport requirements; and the conduct of post-harvest monitoring to ensure GMOs are destroyed.

Conclusions of the RARMP

The risk assessment concludes that this limited and controlled release of GM canola and Indian mustard lines containing genes for herbicide tolerance and a hybrid breeding system poses **negligible** risks to the health and safety of people and the environment as a result of gene technology.

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

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Abbreviations

barnase	Gene derived from *Bacillus amyloliquefaciens*
BARNASE	Protein product from *barnase* gene conferring male sterility
barstar	Gene derived from *Bacillus amyloliquefaciens*
BARSTAR	Protein product from *barstar* gene conferring restored fertility
ANZFA	Previous name for Food Standards Australia New Zealand
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
CCI	Commercial confidential information
CFIA	Canadian Food Inspection Agency
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAD	DDBJ Amino acid sequence Database
DDBJ	DNA Database of Japan
DIR	Dealing involving Intentional Release
DNA	Deoxyribonucleic acid
EFB	European Federation of Biotechnology
EMBL	European Molecular Biology Laboratory
FAO	Food and Agriculture Organization of the United Nations
Farrp	Food Allergy Research and Resource Program
FDA	Food and Drug Administration
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
GenBank	Genetic sequence databank
GM	Genetically Modified
GMAC	Genetic Manipulation Advisory Committee
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectares
kDa	1000 Dalton, unit of relative molecular mass
kg	Kilogram
Km	Kilometres
LD_{50}	Amount of a substance given in a single dose that causes death in 50% of a test population of an organism
m	Metre
mg	Milligram
mL	Millilitre
µg	Microgram
µM	Microliter
MS	Male sterile
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NRL-3D	National Laboratory's Protein Data Bank
NSW	New South Wales
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
PC2	Physical containment level 2
PIR	Protein Information Resource

RARMP	Risk Analysis and Risk Management Plan
Rf	Restored fertility
SA	South Australia
SDAP	Structural Database of Allergenic Proteins
T-DNA	Transfer deoxyribonucleic acid
TGA	Therapeutic Goods Administration
US FDA	United States Food and Drug Administration
USA	The United States of America
WHO	World Health Organisation

Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has decided to issue a licence (DIR 069/2006) to Bayer CropScience Pty Ltd (Bayer) for dealings involving the intentional release of genetically modified (GM) canola and Indian mustard lines into the environment, on a limited scale and under controlled conditions.

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

The Regulator's *Risk Analysis Framework* explains the approach used to evaluate licence applications and to develop the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of her decisions[1].

This RARMP for DIR 069/2006 has been finalised in accordance with the gene technology legislation. Matters raised in the consultation process regarding risks to the health and safety of people or the environment from the proposed dealings were taken into account by the Regulator in deciding to issue a licence and the licence conditions that have been imposed.

Application

Project Title:	Limited and controlled release of GM herbicide tolerant hybrid *Brassica napus* and *Brassica juncea*[2]
Applicant:	Bayer CropScience Pty Ltd
Common name of the parent organisms:	Canola and Indian mustard
Scientific name of the parent organisms:	*Brassica napus* (L.) oleifera Metzg. *Brassica juncea* (L.) Czern and Coss.
Modified trait(s):	Herbicide tolerance and hybrid breeding system
Identity of the gene(s) responsible for the modified trait(s):	• Hybrid breeding system - *barnase* (male sterility) and *barstar* (fertility restorer) genes derived from the bacterium *Bacillus amyloliquefaciens* • Herbicide tolerance trait - *Details declared Commercial Confidential Information (CCI)*

[1] More information on the assessment of licence applications and copies of the *Risk Analysis Framework* are available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <http:://www.ogtr.gov.au/> and <http://www.ogtr.gov.au/pdf/public/raffinal2.2.pdf>, respectively.

[2] The title of the licence application submitted by Bayer is *Evaluation of herbicide tolerant hybrid* Brassica napus *and herbicide tolerant hybrid* Brassica juncea *lines.*

Proposed Sites:	A maximum of 42 sites (up to 8 sites per winter and 6 sites per summer season) between 2007 and 2010. ***Shires for Winter trial sites (2007-2009):*** New South Wales: Coolamon, Greater Hume, Lockhart, Junee, Wagga Wagga and Narrandera South Australia: Kingston, Mount Gambier, Naracoorte/Lucindale, Grant, Robe, Tatiara, and Wattle Range Victoria: Ararat, Corangamite, Hindmarsh, Glenelg, Horsham, Moyne, Northern Grampians, Pyrenees, Southern Grampians, Warrnambool, and Yarriambiack ***Shires for Summer trial sites (2007-2010):*** South Australia: Kingston, Mount Gambier, Naracoorte/Lucindale, Grant, Robe, Tatiara, and Wattle Range Victoria: Ararat, Glenelg, Moyne, Northern Grampians, Southern Grampians, and Warrnambool
Proposed Release Size:	A maximum of 252 hectares comprising up to 42 sites of no more than 6 ha.
Proposed Release Dates:	April 2007 to May 2010

Bayer applied for a licence to release GM canola and Indian mustard lines containing introduced genes for herbicide tolerance and a hybrid breeding system into the environment, on a limited scale and under controlled conditions. The trial will take place on a maximum of 252 hectares, comprising up to 42 sites of no more than 6 ha, over 6 seasons between 2007 and 2010. Up to 8 sites per winter and 6 sites per summer season may be used. Potential sites have been identified in 24 shires in New South Wales, South Australia and Victoria.

The GM canola and Indian mustard lines contain either the *barnase* gene or *barstar* gene which confer male sterility (MS) or restore fertility (Rf), respectively, and comprise Bayer's novel hybrid breeding system. Conventional breeding between genetically modified MS and Rf lines results in GM hybrid lines with restored fertility. Bayer intends to release genetically modified MS, Rf and hybrid lines of canola and Indian mustard. All GM canola and Indian mustard lines are herbicide tolerant.

In accordance with the provisions of section 185 of the *Gene Technology Act 2000*, Bayer sought and received approval for details of the trait for herbicide tolerance, including gene constructs and plasmid maps, precise arrangement of the regulatory sequences and data on molecular characterisation to be declared Confidential Commercial Information (CCI). The CCI was made available to the various prescribed experts and agencies that are consulted on the preparation of all RARMPs for DIR applications.

The purpose of the trial is to evaluate agronomic traits such as herbicide tolerance, germination efficiency, and flowering times in the GM canola and Indian mustard lines compared to conventional non-GM canola and Indian mustard, as well as previously approved GM InVigor® canola lines.

Seed of the GM canola and Indian mustard lines will be imported from Canada. Seeds collected during the trial may be shipped back to Canada for further trait evaluation. Bayer envisages that seeds from promising lines may be further assessed in future seasons in Australia (subject to further approvals).

Bayer proposed a number of measures to limit the spread and persistence of the GMO and the introduced genetic materials that have been considered during the evaluation of the application. None of the GM plant materials, or their by-products, will be used for stock feed or human food.

Risk assessment

The risk assessment considered information contained in the application, previous GM canola and Indian mustard assessments, current scientific knowledge, and issues relating to risks to human health and safety and the environment raised in submissions received from consultation with a wide range of prescribed experts, agencies and authorities on the application (summarised in Appendix B of the RARMP). No new risks to people or the environment were identified from the advice received on the consultation RARMP. However, feedback on the consideration of previously raised issues enabled their clarification in the final RARMP.

Two submissions were received from the public on both the application and the consultation RARMP. Summaries of this advice and how it was considered are provided in Appendices C and D of the RARMP, respectively.

A reference document, *The Biology and Ecology of Canola* (Brassica napus) 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>.

The hazard identification process considered the circumstances or events by which people or the environment may be exposed to the GMOs, GM plant materials, GM plant by-products, the introduced genes, or products of the introduced genes.

A hazard (source of potential harm) may be an event, substance or organism. A risk is identified when a hazard is considered to have some chance of causing harm. Those events that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process

Nineteen events were identified and assessed whereby the proposed release of the GM canola and Indian mustard lines might give rise to harm to people or the environment.

These 19 events included consideration of whether 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 plants, or produce unintended changes in their biochemistry or physiology. In addition, consideration was given to the opportunity for gene flow to other organisms, and its effects if this occurred.

All events were characterised in relation to both the magnitude and probability of harm in the context of the controls proposed by the applicant to limit the spread and persistence of the GMOs in both time and space. This detailed consideration concluded that none of the 19 events gave rise to an identified risk that requires further assessment. The principal reasons comprise:

- the scale of the trial is limited in both area and duration
- containment, monitoring and disposal measures proposed by the applicant to limit the spread and persistence of GM canola and Indian mustard plants
- none of the GM plant materials or products from the GM plants 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 allergenicity from these proteins
- limited capacity of the GM canola and Indian mustard lines to spread and persist in natural ecosystems or undisturbed areas
- limited ability and opportunity for the GM canola and Indian mustard lines to transfer the introduced genes to sexually compatible species or other organisms.

Therefore, as no risks to the health and safety of people, or the environment were identified from the limited and controlled release of the GM canola and Indian mustard lines, the level of risk is considered to be **negligible**.

Risk management

A risk management plan builds upon the risk assessment to consider whether any action is required to mitigate the identified risks, and what can be done to protect the health and safety of people and the environment.

As none of the 19 events that were characterised in the risk assessment process are considered to give rise to an identified risk that requires further assessment, the level of risk to human health and safety and the environment from the release of the GM canola and Indian mustard lines is considered to be **negligible.**

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial with no present need to invoke actions for their mitigation. However, containment measures have been imposed to restrict the release to the locations, size and duration requested by the applicant, as these were important parameters in establishing the context for assessing the risks.

Licence conditions to manage this limited and controlled release

A number of licence conditions have been imposed to limit and control the release, including requirements to:

- surround each site with a 50 m monitoring zone from which related weed species and crop plants would be removed prior to flowering, as well as one of the following measures:
  - *maintain a 1 km isolation zone between the site and any other Brassica crop,* ***or***
  - *surround the site with a 15 m pollen trap (non-GM canola or Indian mustard) and 400m isolation zone from any other Brassica crop,* ***or***
  - *surround the site with a 400 m isolation zone from any other Brassica crop if the GMOs at the site are all GM male sterile canola or Indian mustard,* ***or***
  - *surround the site with a 400m isolation zone from any other Brassica crop if all GMOs at the site are covered with cages, tents or selfing bags*[3]
- harvest and store canola and Indian mustard seed from the release separately from commercial Brassica crops
- not permit canola or Indian mustard seed or other materials from the release to be used in human food or animal feed

[3] The purpose of using insect proof cages, tents or selfing bags is to maximise seed purity but as they also reduce pollen flow, the spread and persistence of the introduced genes will also be limited.

- treat all pollen trap plants as if they are GM plants
- destroy all GM plant materials not required for further analysis by methods approved by the Regulator
- following harvest, clean each site, monitoring zone and equipment to remove all GM plant materials
- following cleaning, encourage germination of GM seed through the use of 'light' tillage of each site
- monitor for, and destroy any, volunteer GM *B. napus* and *B. juncea* that may occur at each site for 24 months after harvest and thereafter until the site is free of volunteers for a continuous 12 month period.

The Regulator has issued guidelines and policies for the transport, supply and storage of GMOs (*Guidelines for the transport of GMOs, June 2001; Policy on transport and supply of GMOs, July 2005*). Licence conditions based on these guidelines and policies have also been imposed to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework. The Regulator sought input on the preparation of the RARMP from other agencies that also regulate GMOs or GM products including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS), National Health and Medical Research Council (NHMRC) and Australian Quarantine Inspection Service (AQIS). Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies[4].

FSANZ is responsible for human food safety assessment, including GM food. The applicant does not intend any material from the GM canola and Indian mustard lines proposed for release to be used in human food. Accordingly the applicant has not applied to FSANZ to evaluate any materials from the trial for use in human food. FSANZ approval would need to be obtained before such materials could be used in human food.

The APVMA is responsible for the use and safety of herbicides in Australia. The canola and Indian mustard lines proposed for release have been genetically modified for herbicide tolerance. Bayer states that the extent of herbicide application to herbicide tolerant GM lines would not exceed 5 ha nationally per annum. Hence it would be authorised under the APVMA's general, small scale trial permit (APVMA Permit 7250).

Bayer has indicated they intend to obtain a permit from AQIS to import seed of the GM canola and Indian mustard lines.

Approval may also be required from the State and Territory Governments that have introduced legislation to delay the commercial introduction of GM canola due to concerns regarding possible market impacts.

[4] 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/pdf/public/raffinal2.2.pdf>.

Identification of issues to be addressed for future releases

The risk assessment identified additional information that may be required to assess an application for a larger scale trial, reduced containment measures, or a commercial release of any of these GM canola and Indian mustard lines. These would include:

- molecular characterisation of the introduced genetic materials, genotypic stability, and expression levels of the introduced genes in the GM canola and Indian mustard lines
- data on the potential toxicity of plant material from the GM canola and Indian mustard lines including levels of known endogenous toxins
- data on the viability of Indian mustard pollen
- the level of pollen mediated gene flow between both canola and Indian mustard and closely related plants in Australia
- biochemical, physiological and agronomic characteristics of the GM canola and Indian mustard lines indicative of weediness including measurement of germination, seed dormancy, tolerance to environmental stresses (eg heat, drought or disease) and reproductive capacity (eg growth rate and window of flowering) compared to the non-GM parent lines.

Conclusions of the RARMP

The risk assessment concludes that the limited and controlled release of GM canola and Indian mustard lines containing genes for herbicide tolerance and a hybrid breeding system poses **negligible** risks to the health and safety of people and the environment posed by, or as a result of, gene technology.

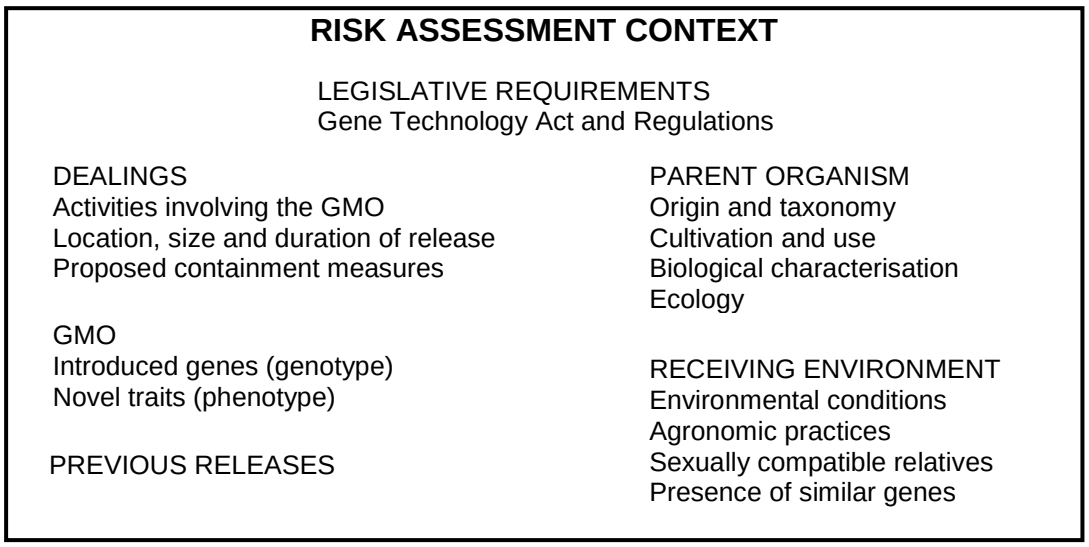
The risk management plan concludes that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to contain the release to the size, duration and locations requested by the applicant, as these were important parameters in establishing the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

Chapter 1 Risk assessment context

Section 1 Background

1. This Chapter describes the parameters within which risks that may be posed to the health and safety of people and the environment by the proposed release are assessed. These include the scope and boundaries for the evaluation process required by the gene technology legislation[5], details of the intended dealings, the GMO(s) and parent organism(s), previous approvals and releases of the same or similar GMOs in Australia or overseas, environmental considerations and relevant agricultural practices. The parameters for the risk assessment context are summarised in Figure 1.

Figure 1 Components of the risk context considered during the preparation of the Risk Assessment



2. Sections 49 to 51 of the *Gene Technology Act 2000* (the Act) outlines the matters, which the Regulator must take into account, and who she must consult with, in preparing the RARMPs that form the basis of her decision on licence applications.

3. For this application, establishing the risk assessment context includes consideration of:

- the size, duration and locations of the trial proposed by the applicant
- containment measures for the GMOs proposed by the applicant
- characteristics of the parent organisms
- the nature and effect of the genetic modifications
- the environmental conditions in the locations where the release may occur
- relevant agricultural practices

[5] The legislative requirements and the approach taken in assessing licence applications are outlined in more detail at <http://www.ogtr.gov.au/ir/process.htm> and in the *Risk Analysis Framework* (OGTR 2005) <http://www.gov.au/pdf/raffinal2.2.pdf>.

- presence of related conventional non-GM or GM crops and other plant species
- presence of the introduced or similar genes and their encoded proteins in the environment
- any previous releases of these or other GMOs relevant to this application.

4. Initial consideration of the application under section 49 of the Act determined that public consultation was not required for the preparation of the consultation version of the RARMP. In accordance with section 50 of the Act, the Gene Technology Technical Advisory Committee (GTTAC), State and Territory governments, prescribed Australian Government agencies, the Minister for Environment and Water Resources and the local councils where the release may take place were consulted on matters relevant to the preparation of the consultation RARMP. This advice, and where it was taken into account, is summarised in Appendix B. Two submissions from members of the public were also received. This advice and its consideration is summarised in Appendix C.

5. In accordance with section 52 of the Act, the Regulator notified the public when the consultation version of the RARMP had been prepared and invited written submissions Two submissions from the public and how they were considered is summarised in Appendix D. Advice on the RARMP was also sought from the same experts, agencies and authorities as before. None of the latter raised any new issues relating to risks to human health and safety and the environment that required further consideration. However, feedback on the consideration of some previously raised issues enabled their clarification in the final RARMP.

Section 2 The application

6. Bayer proposes to release GM canola and Indian mustard lines into the environment under limited and controlled conditions. The GM lines proposed for release contain introduced genes for herbicide tolerance and a hybrid breeding system.

2.1 The proposed location, size and duration

7. The release is proposed to take place on a maximum of 252 ha, comprising up to 42 sites of no more than 6 ha between 2007 and 2010. Up to 8 sites per winter and 6 sites per summer season are proposed. Potential sites have been identified in 24 shires in New South Wales, South Australia and Victoria.

2.2 The proposed dealings

8. The aims of the proposed trial are to evaluate agronomic traits such as herbicide tolerance, germination efficiency, and flowering times in the GM canola and Indian mustard lines compared to non-GM canola and Indian mustard as well as previously approved GM InVigor® canola lines.

9. Seed of the GM canola and Indian mustard lines proposed for release will be imported from Canada. Seeds collected from the trial would be shipped back to Canada for further trait evaluation. Bayer envisages that seeds from promising lines may be assessed in future seasons in Australia (subject to further approvals).

2.3 The proposed measures to limit the spread and persistence of the GMOs

10. Bayer has proposed a number of measures to limit the spread and persistence of the GM canola and Indian mustard lines into the environment. These are taken into account in the risk assessment context (this Chapter) and their suitability for limiting the release to the location, size and duration proposed by the applicant is evaluated in Chapter 2.

11. Bayer has proposed the following containment measures:

- surround each site with a 50 m monitoring zone from which related weed species and crop plants would be removed prior to flowering, as well as one of the following measures:
  - *maintain a 1 km isolation zone between the site and any other Brassica crop,* ***or***
  - *surround the site with a 15 m pollen trap (non-GM canola or Indian mustard) and 400m isolation zone from any other Brassica crop,* ***or***
  - *surround the site with a 400 m isolation zone from any other Brassica crop if the GMOs at the site are all GM male sterile canola or Indian mustard,* ***or***
  - *surround the site with a 400m isolation zone from any other Brassica crop if the all GMOs at the site are covered with cages, tents or selfing bags*[6]
- not permit canola or Indian mustard seed or other materials from the release to be used in human food or animal feed
- cleaning of equipment and destroying all GM plant materials not required for further analysis by methods approved by the Regulator
- monitoring for, and destroying any, volunteer GM *B. napus* and *B. juncea* that may occur in the release area for 3 years on a monthly basis after completion harvest
- specific containment, transport and storage conditions in accordance with OGTR guidelines.

Section 3 The parent organisms

12. The parent organisms are *B. napus* (L.) oleifera Metzg. and *B. juncea* (L.) Czern. and Coss., commonly known as canola and Indian mustard, respectively. Both canola and Indian mustard are exotic to Australia and are grown as agricultural crops mainly in New South Wales, Victoria, South Australia and Western Australia. Toxicity and allergenicity, weediness and potential for gene flow of canola and Indian mustard are presented below. More detailed information on canola can be found in the document, *The Biology and Ecology of Canola* (Brassica napus), which was produced to inform the risk assessment process for licence applications involving GM canola. This document is available at <http://www.ogtr.gov.au>.

3.1.1 Toxicity and Allergenicity

Canola

13. *Brassica napus* seed naturally contains erucic acid and glucosinolate which are toxic to humans and other organisms. The term canola refers to those varieties of *B. napus* that meet specific standards on the level of erucic acid and glucosinolates. These cultivars must yield oil low in erucic acid and meal low in glucosinolates and are often referred to as double low varieties.

14. Canola has become more important to the western world, through breeding for better oil quality and improved processing techniques (OECD 1997). Edible oil was first extracted in Canada in 1956 (Colton & Potter 1999). Canola is now grown primarily for its seeds, which yield between 35 % to over 45 % oil. Its main use is as a cooking oil, but it is also commonly used in margarine.

15. Canola meal is produced as a by-product during the extraction of oil from canola seed and is widely used as a high protein feed source in animal nutrition. Full fat canola seed may also

[6] The purpose of using insect proof cages, tents or selfing bags is to maximise seed purity but as they also reduce pollen flow, the spread and persistence of the introduced genes will also be limited.

be used directly as animal feed (Roth-Maier 1999). Industry standards require canola meal to be low in glucosinolates (total glucosinolates of 30 μmoles/g) in toasted oil free meal (Organisation for Economic Co-operation and Development (OECD) 2001). The maximum level for erucic acid is 2% in the oil fraction (CODEX 2001).

16. No allergic reactions to fats (including canola oil) have been reported in people. The processing of canola seed is expected to remove all traces of protein from the oil (ANZFA 2001a) and given the similarity of the crops, this is expected to be the case for Indian mustard oil as well. Occupational exposure to canola pollen (Chardin et al. 2001; OGTR 2002), canola dust (Suh et al. 1998) and canola flour (Monsalve et al. 1997; Alvarez et al. 2001) have been implicated in allergic reactions in people and a number of putative allergens have been characterised, including seed storage proteins (Monsalve et al. 1997). It is important to note that these findings relate to non-GM canola and that canola seed meal or flour is not considered suitable for human food. Canola oil is the only fraction used for human food. As a quality control measure, no detectable protein is allowed to be present in canola oil (CODEX 2001).

Indian Mustard

17. Indian mustard has a long history of use as a staple food particularly in Asia and parts of Europe. Different varieties of Indian mustard have been grown as vegetables (edible foliage) and the seeds have been used for oils and as a condiment. The aroma and flavour of mustard comes from glucosides in oils contained within the seeds. The compounds responsible for the pungency are predominantly the breakdown products of propenyl (allyl) glucosinolates along with smaller amounts of allyl cyanide and carbon disulfide (Oram et al. 1999).

18. Like canola, Indian mustard seeds naturally contain high levels of glucosinolates and erucic acid, both of which are toxic. There is no evidence of any direct toxicity associated with the use of Indian mustard seeds as food crops for people (Kramer et al. 1983; Monsalve et al. 2001). Oils containing high levels of erucic acid, used as a staple for cooking and in food products since ancient times, do not appear to have any nutritional or health problems associated with their use (Kramer et al. 1983; Monsalve et al. 2001).

19. Traditionally in India, China and Eastern Europe, high toxicant levels have been accepted in oils, but lower levels of both erucic acid and glucosinolates are required for oilseed production in Australia and other countries. This has lead to conventional breeding programs to develop Indian mustard lines with low levels of toxicants (and higher levels of oil) known as ‘canola quality juncea’ or ‘juncea canola’.

20. Indian mustard meal is produced as a by-product during the extraction of oil from the seed and may be used as a high protein feed source in animal nutrition. The use of conventional Indian mustard meal as stock feed is limited by the same industry standards that apply to canola and require meal to be low in glucosinolates and the oil low in erucic acid.

Toxicity Studies

21. In toxicity tests on laboratory animals, diets high in erucic acid were associated with fat infiltration into myocardial tissue (the thick muscular wall of the heart) and retarded growth rates (Kramer et al. 1983; Monsalve et al. 2001). When meal from canola and other Brassica oilseeds with high levels of glucosinolates were fed to non-ruminant animals, the glucosinolates caused reduced food conversion efficiency (Bell 1984). Canola contains goitrogenic compounds (able to suppress the function of the thyroid) such as thiocyanate precursors (see review in Bell 1984).

22. Ruminants are known to eat mustard seeds and meal avidly, and if consumed in excess, these may kill the animals, presumably due to the effect of propenyl isothiocyanate (a glucosinolate) on the lining of the stomach and the pericardium (the double walled sac which contains the heart) (Oram et al. 2005).

23. Allyl isothiocyanate (synonym of propenyl isothiocyanate) has also been shown to cause increased embryonal death and decreased foetal weight in rats. This does not appear to be a significant component of canola (Bell 1984) and does not occur in the mustard seed. However, when ground mustard seeds are mixed with water or other liquids, or when chewed, a chemical reaction between the enzyme myrosinase and the glucosinolate sinigrin from the seeds of black mustard (*B. nigra*) or Indian mustard (*B. juncea*) leads to the production of allyl isothiocyanate (http://en.wikipedia.org/wiki/Allyl_isothiocyanate). Sinigrin is also found in other *Brassica* such as brussels sprouts and broccoli.

24. Allergy to the condiment mustard, typically a mixture of *B. juncea* and *Sinapis alba* (white mustard), has been reported as being the fourth most important food allergy in children after eggs, peanuts and cow's milk (Rance et al. 2000). Other reports indicate it accounts for 1.1% of food allergies in children (Morisset et al. 2005) and that clinical symptoms of allergy to mustard in children are not as severe as in adults (Figueroa et al. 2005).

25. A major allergen of Indian mustard, BRA J 1[7], has been identified as a seed storage protein from the 2S albumin family. Its structure is closely related to that of the allergen of *Sinapis alba*. BRA J 1 is thermostable and resistant to digestion by trypsin and degradation by other proteolytic enzymes (Figueroa et al. 2005), which are two of the characteristics of known food allergens (Metcalfe 1996, FAO 2001).

26. Although Indian mustard has been grown for centuries, a search of the literature did not reveal any reports of allergenicity presented by occupational or even incidental exposure to Indian mustard pollen. However, the potential for allergenicity presented by occupational or incidental exposure would likely be similar to that of closely related canola pollen discussed above.

27. Flour and seed meal from Indian mustard and canola are considered unsuitable for human food. As a quality control measure, no detectable protein is permitted in canola oil and the same quality standards are applicable to Indian mustard oil (Codex 2001). No allergic reaction to fats (including canola oil) has been reported in humans (refer DIR32/2002 RARMP).

3.1.2 Weediness

28. There are weedy relatives of canola and Indian mustard in Australia. Of the 160 species of *Brassicaceae* present in the southern Australian cropping zone, several species are crops of economic importance and several species are recognised as important weeds, namely *Raphanus*, *Sinapis* and some *Brassica* species including *B. rapa*, *B. juncea* and *B tournefortii*. In Australia, other important cropping weeds from the *Brassicaceae* family include *Hirschfeldia incana*, *Diplotaxis* spp. and *Sisymbrium* spp. Of the weeds listed, *B. juncea* has the most restricted distribution, occurring only in a few areas of South Australia and three other States (Rieger et al. 1999).

29. Indian mustard and canola have a number of 'weedy traits'. Indian mustard may be more tolerant to heat and moisture stress than canola which could potentially result in increased persistence. It is however, less prone to pod shattering than canola. Pod shattering at seed

[7] Allergens are designated according to the accepted taxonomic name of their source as follows: the first three letters of the genus, space, the first letter of the species, space, and an Arabic number (http://www.allergen.org/Pub.htm). Thus, BRA J 1 refers to the first allergen identified from *Brassica juncea*.

maturity and during harvest would help to disperse seed, thus increasing the spread and persistence of the species. Indian mustard has the same weediness traits of induced seed dormancy, self- and cross-pollination and unspecialised pollinators as canola (Oram et al. 2005), but also like canola, it is considered to be a poor competitor and does not establish well in undisturbed areas (Oram et al. 2005; CFIA 2005) such as natural ecosystems (Groves et al. 2003).

30. 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. Losses of up to 10,000 seeds/m^2 have been measured (Lutman 1993) often resulting in high densities of these plants occurring as weeds ('volunteers') in subsequent crops (Legere et al. 2001).

31. As with all crops harvested on a large scale, some seed may escape harvest and remain in the soil until the following season when it germinates either before or following seeding of the succeeding crop. In some instances the volunteers may compete with the seeded crop and warrant chemical and/or mechanical control. Volunteers can also be expected to be found outside the planting sites (eg along roadsides and around storage facilities) as a result of transportation of seed out of fields (eg in farm equipment) and spillage during transport.

Weediness: Canola

32. Canola is considered a major weed in agricultural ecosystems in Australia (Groves et al. 2003) and a minor weed in Canada (Canadian Food Inspection Agency 1994) and the USA (Weed Science Society of America 1992). Surveys have shown that canola occurs as a volunteer weed in up to 10% of cereal crops in southern Australia (Lemerle et al. 1996) and similar levels have been reported in Canadian cereal crops (Thomas et al. 1998). Canola is a plant of disturbed habitats (mainly agricultural fields) and will take advantage of disturbed land (Salisbury 2002c).

33. In 2000/2001, a rating system was applied to naturalised, non-invasive species in both natural and agricultural systems based upon information supplied by Australian States and Territories (Groves et al. 2003). As a result, weeds were described as naturalised[8] and were defined as environmental or agricultural weeds[9] depending on how they impact either ecosystem. The weeds were further categorized based on their status within each ecosystem on a scale from 0 (indicating naturalised, but the population no-longer existing or removed) to 5 (indicating naturalised and known to be a major problem at four or more locations within a State or Territory).

34. *Brassica napus* is a classified as a category 2 weed in natural ecosystems and category 5 weed in agricultural ecosystems. Category 2 weeds are naturalised and known to be a minor problem warranting control at three or fewer locations within a State or Territory and described as primarily a weed of agricultural or disturbed areas (Groves et al. 2003). Wheat, which is often grown in rotation with canola, is a category 2 weed in natural ecosystems, a category 3 weed in agricultural ecosystems and is also described as primarily a weed of agricultural or disturbed areas (Groves et al. 2003).

35. Canola seed can be dispersed to neighbouring non-agricultural areas by mechanisms such as strong winds blowing canola windrows across or off a field or seed may be dispersed with

[8] Naturalised non-native species may be defined as those that have been introduced, become established and that now reproduce naturally in the wild, without human intervention (Groves et al. 2003)

[9] Environmental weeds are naturalised non-native species that have invaded non-agricultural areas of natural vegetation and are presumed to impact negatively on native species diversity or ecosystem function. Environmental weeds are distinguished from agricultural weeds by the ecosystem they impact.

straw and chaff during mechanical harvest. However, canola is a poor competitor and is not regarded as an environmentally hazardous colonising species. Unless the habitat is regularly disturbed, or seed replenished from outside, canola will be displaced by other plants (Salisbury 2002c).

36. Populations of canola can be found on roadside verges, in field margins and along railway lines in all countries where it is grown. An Australian survey encompassing a total of 4000 km of road and 400 observations recorded incidences of canola plants growing within 5 m of the roadside (Agrisearch 2001). The incidence of canola in the major canola growing districts was as follows: southern NSW (31 %), Victoria (13 %), SA (9 %), WA (20 %) and Tasmania (14 %). The occurrence of predominantly isolated plants suggests they had not originated from seed derived from plants grown the previous season, but resulted from individual seeds being dropped during transportation. Dignam (2001) surveyed 103 local councils across Australia and evidence of canola was present in 30 % of the councils surveyed. In the survey, the density of roadside canola populations was mostly low. Only 5 % of councils and 4 % of road and rail authorities surveyed, indicated canola was present in large numbers.

Weediness: Indian mustard

37. *Brassica juncea* has previously been listed as a 'noxious' weed in some jurisdictions of Australia. However, evidence provided by breeders has resulted in a down-grading of this categorisation to its current status as a naturalised, non-native species (pers. comm., P. Salisbury, 2005). Based on the weed classification system discussed above, *B. juncea* is a category 3 weed in natural ecosystems, a category 5 weed in agricultural systems and described as primarily a weed in agricultural or disturbed areas. A category 3 weed is naturalised and known to be a minor problem warranting control at 4 or more locations within a State or Territory. For comparison, barley falls in the same categories for natural and agricultural weeds and is also primarily a weed in agricultural or disturbed areas (Groves et al. 2003).

38. Several thousand hectares of conventional Indian mustard has been grown in south eastern Australia for approximately 25 years as part of a small industry, which produces cold-pressed mustard oils and providing seed for the specialist condiment market. In a long term study, Indian mustard grown near Harden, NSW, it has been reported that Indian mustard seedlings volunteer less frequently in subsequent crops than canola (Oram et al. 2005).

39. Similar to canola, Indian mustard occurs in disturbed habitats along roadsides, railway lines and in field margins in regions where it has been cultivated (Oram et al. 2005). In Australia, feral populations of canola rarely persist (Salisbury 2002a; Salisbury 2002c; Brooks et al. 2003; Baker & Preston 2004) and observations (above) suggest that Indian mustard is less likely than canola to volunteer in subsequent crops or to persist as a feral population (Oram et al. 2005).

3.1.3 Potential for gene transfer

40. Canola and Indian mustard are mainly self-pollinating with pollen that is relatively heavy and sticky. Insects, particularly bees, are the primary cross-pollinators. In Australia, honeybees (*Apis mellifera*) are believed to be the main insect responsible for transfer of canola and Indian mustard pollen. Studies have shown that a large proportion (up to 80%) of bee flights are less than 1 m in distance, with the majority of pollen being transported less than 5 m by bees (e.g.(Cresswell 1999); (Ramsay et al. 1999); (Pierre 2001). Occasionally however, bees may travel much further and studies have measured bee flight distances of 1 to 2 km (Eckert 1933), up to a maximum distance of 4 km (Ramsay et al. 1999); (Thompson et al. 1999). Nonetheless, the highest rate of cross-pollination occurs with close proximity and

in situations where there is physical contact with neighbouring plants. Unless precautions were taken, it is expected that there would be some gene transfer between the GM canola or GM Indian mustard and non-GM lines or other sexually compatible species that may be grown at the site as part of the trial.

41. Pollen viability varies with environmental conditions, particularly temperature and humidity. Under controlled conditions in the laboratory, canola pollen can remain viable for between 24 hours and one week (Mesquida & Renard 1982) and under natural conditions canola pollen viability gradually decreases over 4-5 days (Ranito-Lehtimäki 1995). Similar studies on Indian mustard have not been reported, but the applicant stated that pollen viability of *B. juncea* is 3 days.

42. Outcrossing rates of 10 to 50% have been reported for canola, so it is likely that gene flow will occur between populations of canola. The transfer of genes between canola populations is well documented (Paul et al. 1995; Scheffler et al. 1995; Downey 1999). There are fewer studies regarding outcrossing rates for Indian mustard. Under Canadian field conditions, outcrossing rates for Indian mustard were as high as 29% (Rakow & Woods 1987). In the same study, out-crossing rates for *B. napus* were 30%, indicating similar outcrossing rates for both species. In the absence of studies specific to Indian mustard under Australian field conditions, current experimental evidence regarding pollen distribution patterns for canola in Australia, discussed above, may be useful as a guide to likely patterns for Indian mustard.

43. Outcrossing from canola or Indian mustard to sexually compatible species is possible. Canola can outcross to other *B. napus* groups or subspecies (including vegetables such as Swedes, rutabaga, kale), *B. rapa* (including vegetables such as turnip, chinese cabbage, pak choi) and *B. juncea*. Canola is a tetraploid containing two copies of the A and C genomes and is designated as AACC. It shares a common set of chromosomes with *B. rapa* (diploid genome designated AA) and Indian mustard (tetraploid genome designated AABB). Interspecific hybrids between *B. napus* and the other two species have been reported in several countries with backcrossing and gene introgression reported to occur at low frequencies. In contrast, naturally occurring hybrids (under field conditions) between Indian mustard and *B. rapa* have not been reported (Salisbury 2006).

44. Outcrossing from canola or Indian mustard to cultivated vegetables of *B. napus*, or between canola and vegetables of *B. rapa* could also occur provided they are in close proximity and there is synchrony of flowering. 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 or Indian mustard and vegetable crops of *B. napus* and *B. rapa* are unlikely to occur.

45. Hybrids between *B. oleracea* vegetables (cauliflower, broccoli, Brussel sprouts, kohlrabi, etc) and *B. napus* or *B. juncea* have not been reported under natural conditions, and again, these vegetable crops are also harvested prior to flowering (Salisbury 2002a; Salisbury 2006).

46. Naturally occurring hybrids between *B. napus* and weed species in the tribe *Brassiceae*, such as *Raphanus raphanistrum* (wild radish), *Hirschfeldia incana* (Buchan weed) and *Sinapis arvensis* (charlock) have been reported at very low frequencies (Salisbury 2002a). 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 very low frequencies. In addition, after five generations of backcrossing, the herbicide tolerance trait had not been introgressed into the *R. raphanistrum*

or *H. incana* genomes. Hybrids between *B. napus* and *S. arvensis* have been detected at extremely low frequencies and only if *S. arvensis* is the male parent and *B. napus* is male sterile. No hybrids have been reported between *S. arvensis* and *B. napus* when both parents are fully fertile. There have been no reported natural hybrids occurring between *B. juncea* and these three weed species (Salisbury 2002a; Salisbury 2006).

47. Natural hybrids between *B. napus* or *B. juncea* and other weed species in the *Brassiceae* tribe 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* or *B. juncea* and other weed species in tribes other than *Brassiceae* (Salisbury 2002a; Salisbury 2006).

Section 4 The GMOs, nature and effect of the genetic modification

4.1 Introduction to the GMOs

48. The parent plants of the GM canola lines proposed for release were initially, modified by the introduction of genes for herbicide tolerance[10] and either male sterility (MS) or to restore fertility (Rf). Conventional breeding of MS lines (containing the *barnase* gene) with the Rf lines (containing the *barstar* gene) resulted in GM hybrid lines with restored fertility and herbicide tolerance. GM hybrid, MS and Rf canola lines are proposed for release as part of the trial.

49. The GM Indian mustard MS and Rf lines proposed for release were derived from conventional crossing of GM herbicide tolerant canola lines with non-GM Indian mustard varieties in laboratories and greenhouses in Europe. The F_1[11] hybrids have been backcrossed[12] with Indian mustard varieties which increases the genetic proportion of the recurrent parent. Like the GM hybrid canola above, fertile GM hybrid Indian mustard lines were derived through conventional breeding between MS and Rf lines. GM hybrid, MS and Rf Indian mustard lines are proposed for release as part of the trial.

50. The *barnase* gene encodes the BARNASE enzyme (a ribonuclease) and is regulated by an anther specific promoter which limits expression to an early stage of anther development, in a specific cell layer of the anthers (the tapetum cells of the pollen sac). This results in flowers without anthers, preventing pollen production and thus conferring male sterility (Hartley 1989);(Mariani et al. 1990).

51. The *barstar* gene encodes the BARSTAR enzyme (a ribonuclease inhibitor) which is also regulated by an anther specific promoter. In hybrid plants derived from crosses of MS and Rf lines, the BARSTAR enzyme inhibits the BARNASE enzyme enabling normal anther development and pollen production (Hartley 1989); (Mariani et al. 1990); (Mariani et al. 1992). The hybrids are therefore fully fertile and plants display hybrid vigour (enhanced yield associated with crosses between different parental lines).

52. Both the *barnase* and the *barstar* genes are derived from a common soil bacterium, *B. amyloliquefaciens* which is used extensively in the food industry to produce industrial enzymes such as α-amylase (ANZFA 2001a). While some *Bacillus* species have been

[10] Details of the herbicide tolerance trait have been declared as confidential commercial information (CCI).
[11] **F_1 hybrid:** the first generation of a cross between two parents.
[12] **Backcross**: a cross between the heterozygous F_1 (first) generation and either of its parents (in this case *B. juncea*) which increases the proportion of the recurrent parent (*B. juncea*) in the progeny.

implicated in infections to people, *B. amyloliquifaciens* has no known pathogenicity and is used in brewing, bread-making and the food industry as a whole.

53. Short regulatory sequences that control expression of the genes are also present in all the GM canola and Indian mustard lines. These are derived from plants and plant pathogens including a plant virus[13]. However, the sequences derived from plant pathogens comprise only a small part of their total respective genomes, and are not capable of causing disease.

4.1.1 Introduction to hybrid seed production

54. Traditional plant breeding selects for plants with agronomically valuable characteristics but repetitive self-pollination can produce inbred plants that may display lowered fitness or vigour as compared with their non-inbred counterparts. The converse of this, hybrid vigour, can occur when the progeny from crosses of genetically distinct parents outperform the parental lines in yield, increased resistance to disease and enhanced agronomic performance.

55. An historic example of hybrid seed production is hybrid corn seed which is produced by crossing inbred corn lines. The technology to produce hybrid corn seed has been available for farmers in the US for more than 80 years. Corn is monoecious, with separate male and female flowers, which physically facilitates hybrid seed production. Mechanical removal of the male flower (tassel) from one inbred line (female) allows for wind-borne cross pollination from another inbred line (male) and the production of hybrid seed. Mechanical removal of the tassel was no longer required when genes for cytoplasmic male sterility (CMS) and fertility restoration of corn were identified in the 1940's.

56. Cytoplasmic male sterility (CMS) has been identified in many other crops including green beans, sorghum, beet, carrot, *B. napus*, sunflower and wheat. Several CMS lines have been developed in the crop Brassicas through the production of alloplasmic types of cultivated species following wide hybridisation (Malik et al. 1999).

57. The first conventional (non-GM) canola hybrids based on the CMS system were released in Australia in 1988. However, to date, a limitation to the cultivation of these has been that they have not consistently out yielded conventional cultivars sufficiently to justify the higher seed costs.

58. The production of hybrids presents a challenge for plant breeders in crop plants that have both male (stamen which produces pollen) and female (stigma which produces the ovule or egg) reproductive parts on the same plant or in the same flower and are self-fertile. Canola is self-fertile and its flowers normally contain both stamen and stigma.

59. Bayer has utilised gene technology techniques to develop hybrid seed from two distinct parents (refer to Section 4.1 above). The hybridisation system ensures the female lines are pollinated by the desired male lines. Bayer's hybrid system comprises a male sterile (MS) line obtained by the introduction of the *barnase* gene and a fertility restorer (Rf) line obtained by the introduction of the *barstar* gene. Cross pollination through conventional breeding of the MS and Rf lines results in hybrid lines which are fully fertile.

60. GM InVigor® canola hybrids have been reported to demonstrate significant yield advantages of 10 to 25% over open-pollinated cultivars in Australian and Canadian trials (GRDC, 2005; http://www.grdc.com.au/growers/res_upd/ south/s05/potter.htm)

[13] Bayer sought and received approval to have details of the regulatory elements declared as confidential commercial information (CCI) by the Regulator.

4.2 Toxicity and allergenicity of proteins encoded by the introduced genes

4.2.1 The BARNASE and BARSTAR proteins

61. The male sterility and fertility restorer functions (MS/Rf) in this application are identical to those in GM InVigor® canola that have been discussed in detail in the RARMPs for licences DIR 010/2001, 021/2002, 032/2002, and 057/2004. GM InVigor® canola lines have been grown commercially in North America since 1996 and have been approved for commercial release on human health and environmental grounds in Australia[14] (under Licence DIR021/2002).

62. The toxicity and allergenicity of the BARNASE and BARSTAR proteins have been assessed in previous RARMPs, most recently in the RARMP for licence application DIR057/2004 (see also RARMPs listed above). Examination of potential toxicity and allergenicity of BARNASE and BARSTAR was conducted by comparing the amino acid sequences of these proteins with known toxins and allergens.

63. The BLASTP (Standard Protein-Protein Basic Local Alignment Search Tool) program (http://www.ncbi.nlm.nih.gov/BLAST/) using the BLOSUM62 matrix and limited by the term "toxin" was used to compare the amino acid sequence of these proteins to known protein toxins[15]. Neither the BARNASE nor BARSTAR proteins showed significant homology to any know protein toxin. Both proteins are rapidly degraded in simulated gastric juices (0.32% pepsin and acidic pH) with complete protein degradation within five minutes (Van den Bulcke 1997). A feeding study in rabbits fed *ad libitum* diets comparing GM hybrid canola (expressing the same BARNASE and BARSTAR proteins as proposed for release) and non-GM canola (parent line) showed no differences, indicating that the nutritional value of the GM hybrid canola was comparable to the non-GM parental line (ANZFA 2001a). The above results indicate that the introduced *barnase* and *barstar* genes are unlikely to encode protein toxins.

64. The BARNASE and BARSTAR proteins are not known to cause allergic reactions in people. Both genes encoding these proteins are derived from a commonly occurring soil bacterium (*B. amyloliquefaciens*) which is not known to be allergenic. *B. amyloliquefaciens* is used extensively in the food industry to produce industrial enzymes such as α-amylase (ANZFA 2001a). BARNASE and BARSTAR do not have characteristics typical of known protein allergens and have no structural similarity to known food allergens (Van den Bulcke 1997).

65. Epitopes are usually composed of sugars, lipids or amino acids and are part of a molecule to which antibodies bind and therefore a determinant of allergic responses on cells. Identified epitopes of allergenic proteins tend to be between eight and 12 amino acids long and it has been proposed that a match of at least eight contiguous amino acids is required for an immunologically significant sequence identity (Metcalfe et al. 1996). A search for homology of BARNASE and BARSTAR with known allergens was therefore conducted based on detecting shared sequences of eight continuous amino acids; no sequence homologies were detected (Van den Bulcke 1997).

66. A more refined method based on detecting identities of six amino acids with known IgE epitopes has been published (Kleter & Kuiper 2002). The method was applied to the amino

[14] The governments of the major canola growing States and Territories have introduced legislation to delay the commercial introduction of GM canola due to concerns regarding possible market impacts.

[15] Search includes all GenBank + RefSeq Nucleotides + EMBL + DDBJ + PDB sequences (refer to footnote on next page).

acid sequences of proteins introduced into GM plants, including the BARNASE and BARSTAR proteins. No matches with known IgE epitopes were found (Kleter & Peijnenburg 2002), confirming the previous results for these proteins.

67. Neither the GMOs nor any of their products from this trial will be used in human food or animal feed. However, GM InVigor® canola lines containing BARNASE and BARSTAR have been assessed by Food Standards Australia New Zealand (FSANZ) with regard to toxicity. FSANZ concluded that oil derived from GM canola (containing the introduced *barnase* and *barstar* genes) is as safe and nutritionally equivalent to oil derived from non-GM canola varieties (ANZFA 2001a; ANZFA 2001b). The BARNASE and BARSTAR proteins are present in InVigor® canola lines, which have been approved for commercial release in Australia, as well as overseas (see Section 6 this Chapter), and has been grown in North America since 1996 with no adverse effects reported.

68. On the basis of the above information, the BARNASE and BARSTAR proteins are unlikely to be toxic or allergenic to humans or toxic to other organisms.

4.2.2 The herbicide tolerance proteins

69. Similar to the BARNASE and BARSTAR proteins above, examination of potential toxicity and allergenicity of the introduced herbicide tolerance proteins were conducted by comparing the amino acid sequences of the proteins with known toxins and allergens.

70. The BLASTP program and criterion described above for the BARNASE and BARSTAR proteins was used to compare the amino acid sequence of the herbicide tolerance proteins to known protein toxins. The results of the search revealed no significant homology.

71. The criterion used for indicating potential allergenicity was a 35% identity on a window of 80 amino acids with a toxin or allergenic protein, which is below the threshold for allergen epitope screenings suggested by the FAO/WHO (FAO 2001; Codex Alimentarius Commission 2003). The results of the overall homology search[16] showed no identity to any known allergens, but did show homology with similar proteins from various origins (including bacteria and plant species).

72. The threshold suggested for allergen epitope screenings suggested by the FAO/WHO is ideally six contiguous identical amino acids (FAO 2001; Codex Alimentarius Commission 2003). Five matches of six contiguous amino acids to known allergens in the SADP database were identified. Algorithms have been developed to predict antigenicity, ie the antibody binding of peptide sequences such as the six contiguous matches identified above. A commonly used algorithm is that of Hopp and Woods, which predicts which parts of the amino acid sequence has the highest probability to be part of an antigenic determinant (eg. hydrophilic and acidic amino acids) of the protein (Kleter & Peijnenburg 2002). Analysis of the five matches on the ExPASy website (http://us.expasy.org/cgi-bin/protscale.pl) using the

[16] The following reference databases were searched: Uniprot_Swissprot (http://www.ebi.uniprot.org), Uniprot_TrEMBL (http://www.ebi.ac.uk/swissprot/), PIR (Protein Information Resource, http://pir.georgetown.edu/), NRL-3D (National Laboratory's Protein Data Bank, http://srs.wehi.edu.au/srs6bin/cgi-bin/wgetz?-page+LibInfo+-id+56fdt1TPKDN+-lib+NRL3D), DAD (DDBJ Amino acid sequence Database, http://www.ddbj.nig.ac.jp/Welcome-e.html). DDBJ (DNA Data Bank of Japan) is an international nucleotide sequence database in collaboration with EBI/EMBL and NCBI/GenBank. The EMBL Nucleotide Sequence Database (also known as EMBL-Bank) constitutes Europe's primary nucleotide sequence resource. The database is produced in an international collaboration with GenBank (USA) and the DNA Database of Japan (DDBJ). GenBank® is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (Nucleic Acids Research 2006 Jan 1;34(Database issue):D16-20).

Hopp and Woods algorithm indicated that all five had a negative score and thus were unlikely to be antigenic (that is, unlikely to constitute binding sites (epitopes) for IgE immunoglobulins).

73. The above findings indicate that the herbicide tolerance genes do not encode known toxic or allergenic proteins.

4.3 Method of genetic modification

74. Herbicide tolerance and MS or Rf genes were introduced into non-GM canola plants on six different gene constructs carried by *Agrobacterium tumefaciens* (a soil bacterium) to produce the GM canola lines proposed for release. These constructs are 'disarmed' since they lack the genes that encode the tumour-inducing functions of *A. tumefaciens*. The canola plants were genetically modified in Belgium with further development (such as preliminary screening of the GM plants) undertaken in Belgium, Canada and Australia.

75. *A. tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants. Plants can be genetically modified by the transfer of DNA (transfer-DNA or T-DNA, located between specific border sequences on a resident plasmid) from *A. tumefaciens* through the mediation of genes from the virulence region of Ti plasmids. *Agrobacterium*-mediated transformation has been widely used in Australia and overseas for introducing genes and regulatory sequences for their expression into plants.

76. 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).

77. All GM canola lines contain an introduced gene for herbicide tolerance and this trait was used in the selection of GM plants during the initial stages in the laboratory.

78. The GM Indian mustard herbicide tolerant MS and Rf lines were created by crossing the GM canola MS and Rf lines with non-GM Indian mustard plants followed by 3 or 4 backcrosses to the Indian mustard parent. Subsequently, the GM Indian mustard lines have been crossed and backcrossed into other Indian mustard varieties during the development of lines with superior agronomic characteristics.

79. The GM hybrid canola and GM Indian mustard lines were derived from conventional breeding between selected GM male sterile and GM fertility restorer lines.

4.4 Characterisation of the GM canola and Indian mustard lines proposed for release

80. The GM herbicide tolerant canola and Indian mustard lines proposed for release are at an early stage of development. Some of the individual genetic elements such as the *barnase* and *barstar* genes have been trialled in canola and Indian mustard previously, but the proposed release will trial novel combinations of genes. Thus, the GMOs have not been extensively characterised, but available data on the GM lines is presented below. Research requirements for possible future applications are discussed in Chapter 3.

4.4.1 Stability and molecular characterisation

81. After *Agrobacterium*-mediated genetic modification, shoots were regenerated on selective medium under tissue culture conditions. From these shoots, all GM herbicide tolerant canola

plantlets identified for transfer to the glasshouse were analysed for the presence of the introduced gene and copy number by Southern blot hybridisation, using molecular probes specific for each of the six constructs. GM lines containing complex integration patterns (suggesting integration of multiple copies of the introduced genes) were discontinued. Data for GM canola lines containing any one of constructs 2, 3, 4 or 5, crossed with non-GM canola varieties showed a 1:1 segregation pattern suggesting stable transfer and inheritance of the herbicide tolerance trait (Bayer 2006). Data for GM canola lines containing constructs 1 and 6 was not available. Inheritance and stability of the hybrid breeding trait has been demonstrated through three or more generations of backcrossing from the MS and Rf canola lines into the Indian mustard background. As the genes for the hybrid breeding and herbicide tolerance traits are linked, successfully modified plants were identified via herbicide application (Bayer 2006).

82. Bayer anticipates that as the trial progresses, GM lines with superior agronomic traits may be identified for potential commercialisation and detailed molecular analysis would be undertaken at that time.

4.4.2 Characterisation of the phenotype of the GMOs

83. The applicant stated that aside from male sterility and herbicide tolerance, under glasshouse and field conditions there are no obvious altered phenotypes resulting from the expression of the introduced genes. However, further phenotypic observations will be made during the proposed release by comparing the agronomic performance of the GM lines relative to non-GM canola and Indian mustard lines and GM InVigor® canola under field conditions.

84. Expression of the herbicide tolerance trait was determined by the GMOs' tolerance to herbicide application. Expression of the introduced *barnase* and *barstar* genes was determined by lack of anthers in male sterile lines and restored fertility in hybrids between conventional crossing of the MS and Rf lines.

4.4.3 Toxicity and allergenicity of GM canola and Indian mustard plant materials

85. The applicant has stated that the non-GM canola and Indian mustard parent lines contain low levels of two naturally occurring toxicants, erucic acid and glucosinolates. Non-GM canola and Indian mustard pollens are known to cause allergic reaction in people and the non-GM parents of the GM lines proposed for release would be expected to do the same. An extensive search of the scientific literature and publicly available toxin and allergen databases revealed no evidence to suggest that the introduced genes encode proteins that are allergenic or toxic to people or toxic to other organisms (refer Section 4.2 above). On this basis, the GM canola and Indian mustard lines are unlikely to be more allergenic or toxic than non-GM canola and Indian mustard.

Section 5 The receiving environment

86. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes the size, duration and locations of the dealings, any relevant biotic/abiotic properties of the areas where the release would occur; intended agronomic 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 2005).

5.1 Relevant abiotic factors

87. The size and duration of the proposed release is outlined in Section 2 of this Chapter. The proposed release is to occur in up to 24 shires in NSW, SA and Victoria. These shires are in the canola growing regions and have a typical climate for winter and/or summer cultivation in Australia.

88. In Australia canola is mostly grown in winter dominant rainfall environments (between 30^{o}S and 38^{o}S). Rain-fed crops are sown with the onset of significant rain in April or May. Australian canola varieties flower for a 6-week period with crops ripening in late spring or early summer, after a 5 to 7 month growing season. Small areas of canola are sown in late spring - early summer in more temperate regions of Australia. These crops are located in areas that receive reliable rainfall, or have access to irrigation during summer as well as experiencing cool-mild temperatures at flowering. Summer grown canola crops are harvested in early autumn. Indian mustard is reported to be somewhat more tolerant to heat and moisture stress than canola and may be grown in areas receiving lower rainfall than required for canola.

5.2 Relevant agricultural practices

5.2.1 General information

89. The applicant intends to treat the GM canola and Indian mustard plants according to standard agricultural practices used in Australia. Plants would therefore receive applications of water, fertilisers, herbicides, insecticides and other agronomic management practices similar to non-GM canola and Indian mustard plants. One exception is that at some sites physical barriers (selfing bags, or insect-proof cages and tents) would be placed over flower stalks or entire plants to ensure seed purity. Within the cages or tents, Bayer proposes to utilise bees or flies to facilitate pollination.

5.2.2 Use of bees or flies for pollination

90. Bayer has proposed the use of bees (*Apis mellifera*) or flies [sheep blowflies (*Lucilia cuprina*) or eastern golden haired blowfly (*Calliphora stygia*)] for pollination within insect-proof cages or tents at some of the release sites during the summer season. The cages and tents would be covered with a fine insect-proof mesh to keep bees or flies in and other insects out. Cages would be 1 m x 1 m x 1.5 m high. Two sizes of tents would be used, either 6.5 m x 6.5 m x 2 m high or 10 m x 20 m x 2 m high. Frames would be metal pipes with the insect-proof mesh placed over the frames. Tents would have a zipper to allow entry.

91. Up to 30 tents and 250 cages may be used at each of the summer sites. Cages and tents would be placed over the GM canola and Indian mustard lines at least seven days prior to flowering and removed at podding (seed formation).

92. Bees in cages and tents would be given honey and/or sugar as a food source. Flies would only be released into cages. Approximately 50 flies would be released per cage, with up to 4 releases per cage during the flowering season. Approximately 50 bees would be release per cage and one hive with around 5,000 bees would be located in each tent.

93. The worker bees, which leave the hive to gather nectar and pollen, have a life span of 20 to 40 days, whereas the flies proposed for release have a life span of 14 to 21 days[17]. Bees or

[17] *Lucilia cuprina* live approximately 14 to 21 days (Queensland Department of Primary Industries, 2006, http://www2.dpi.qld.gov.au/sheep/10041.html) and *Calliphora stygia* an average of 25-26 days (Hulbert et al. 2004). However, the data for *C. stygia* is from captivity under artificial condition so the life span is likely to be shorter under natural conditions.

flies introduced into the cages would be killed with insecticides or die naturally before the cages are removed (at podding). Bees and bee hives introduced into tents would be removed at podding (at which time pollen would no longer be viable). After removal from the tents, Bayer indicated that the bees would be re-introduced into apiarist hives and the next crops they would forage would be Lucerne or other non-Brassica crops.

5.2.3 Use of herbicides

94. The canola and Indian mustard lines proposed for release have been genetically modified for herbicide tolerance. Bayer has indicated that the GM lines would be challenged with applications of herbicide (in accordance with appropriate APVMA permits where required) to eliminate any off-types and for weed management.

5.3 Presence of related plants in the receiving environment

95. Potential sites for the proposed trial have been identified in twenty four shires in NSW, SA and Victoria. These shires are located in canola and Indian mustard growing areas of Australia. A number GM canola and Indian mustard lines containing genes for herbicide tolerance and/or a hybrid breeding system have previously been approved for limited and controlled release in these areas (see Licences DIR032/2002 and DIR057/2004). GM glyphosate tolerant Roundup Ready® and GM glufosinate ammonium tolerant, hybrid InVigor® canola have been approved for commercial release on human health and environmental safety grounds (Licences DIRs 020/2002 and 021/2002 respectively). However, because of legislation introduced by State and Territories arising from concerns regarding possible market impacts, no commercial planting of either of these crops has occurred to date. There are also two conventionally bred herbicide-tolerant canola varieties currently grown throughout Australia –triazine tolerant and imidazolinone tolerant.

5.4 Presence of the introduced or similar genes and their products in the environment

96. The *barstar* and *barnase* genes are derived from *B. amyloliquefaciens*, and encode an RNase and its inhibitor, respectively, which are ubiquitous in nature and serve many biological functions. Additionally, *B. amyloliquefaciens* is a commonly occurring soil bacterium and is frequently used as a source of industrial enzymes such as α-amylase. The proteins associated with the introduced herbicide tolerance traits and similar proteins are found in various bacteria and plant species and are widely present in the environment (see Section 4.2.2 above).

Section 6 Australian and international approvals

6.1 Australian approvals of the same or similar GM canola and Indian mustard

6.1.1 Previous releases approved by GMAC or the Regulator

97. Bayer has received approval from the Regulator for the limited and controlled release of GM herbicide tolerant canola and Indian mustard lines containing the same hybrid breeding system as the GMOs proposed for the current trial (see Licences DIR 032/2002 and DIR 057/2004, respectively).

98. Several field trials of GM herbicide tolerant *B. napus*, *B. rapa* and *B. juncea* lines containing the hybrid breeding system were also conducted under the former voluntary system including Planned Release (PR) 63; PR85; and PR90.

99. GM herbicide tolerant InVigor® canola also contains the same hybrid breeding system and the Regulator issued Licence (DIR 010/2001) to Bayer for a limited and controlled

release of up to 318 ha on 90 sites over three years in 23 shires in NSW, Vic, WA and SA. GM InVigor® canola was subsequently approved for commercial release under Licence DIR021/2001.

100. To date, no adverse effects on human health and safety or the environment have been reported for any of these releases.

6.1.2 Approvals by other Australian government agencies

101. 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 that may also have to be met in respect of release of GMOs, under Australia's integrated framework, include that of FSANZ, Australian Pesticides and Veterinary Medicines Authority (APVMA) and Australian Quarantine and Inspection Service (AQIS).

102. FSANZ is responsible for human food safety assessment and food labelling, including GM food. The applicant does not intend to use any of the GM canola and Indian mustard plants from the proposed release, or their by-products for stock feed or human food. An approval from FSANZ would be required before oil from the GM lines could be used for human consumption.

103. FSANZ have approved the use of canola oil derived from the male sterile and fertility restorer lines that make up hybrid InVigor® GM canola for use in food in Australia (ANZFA 2001a). FSANZ has determined that refined oil derived from these lines of canola is as safe for human consumption as refined oil derived from conventional canola (non-GM) varieties.

104. The APVMA is responsible for the use and safety of herbicides in Australia. The canola and Indian mustard lines proposed for release have been genetically modified for herbicide tolerance. Bayer states that the extent of herbicide application to herbicide tolerant GM lines would not exceed 5 ha nationally per annum. Hence it would be authorised under the APVMA's general, small scale trial permit (APVMA Permit 7250).

105. Bayer has indicated it intends to obtain a permit from AQIS to import seed of the GM canola and Indian mustard lines proposed for release.

6.2 International approvals

106. The GM herbicide tolerant hybrid canola and Indian mustard lines proposed for release under the current application have been approved for glasshouse and field trials in Canada and Belgium (Bayer 2006) but have not been released commercially in other countries. A number of overseas field trials of GM canola and Indian mustard with introduced genes for hybrid systems and/or herbicide tolerance traits are presented below.

107. During 2000 and 2001, Aventis CropScience in Belgium conducted three field trials of GM Indian mustard with a hybrid breeding system (*gmoinfo.jrc.it/gmp_browse_geninf.asp*).

108. A number of GM InVigor® canola lines containing the *barnase* and *barstar* genes have been approved for growing and consumption in Canada, Japan and USA. Further detail is provided in the RARMP for DIR 021/2002 available at www.ogtr.gov.au or from the OGTR.

109. The Canadian Food Inspection Agency (CFIA) website lists numerous field trials of GM plants with novel traits, including *B. napus* and *B. juncea*, that have been approved in recent years (http://www.inspection.gc.ca/english/plaveg/bio/st/st_06e.shtml). In 2006, 44 out of 188 trials of GM canola and 12 out of 15 trials of GM Indian mustard approved in Canada involved the use of MS and Rf lines.

110. There are 8 field test release permits for GM canola and 1 for GM Indian mustard currently listed on the USDA field test database (*www.isb.vt.edu/CFDOCS/fieldtests1.cfm*).

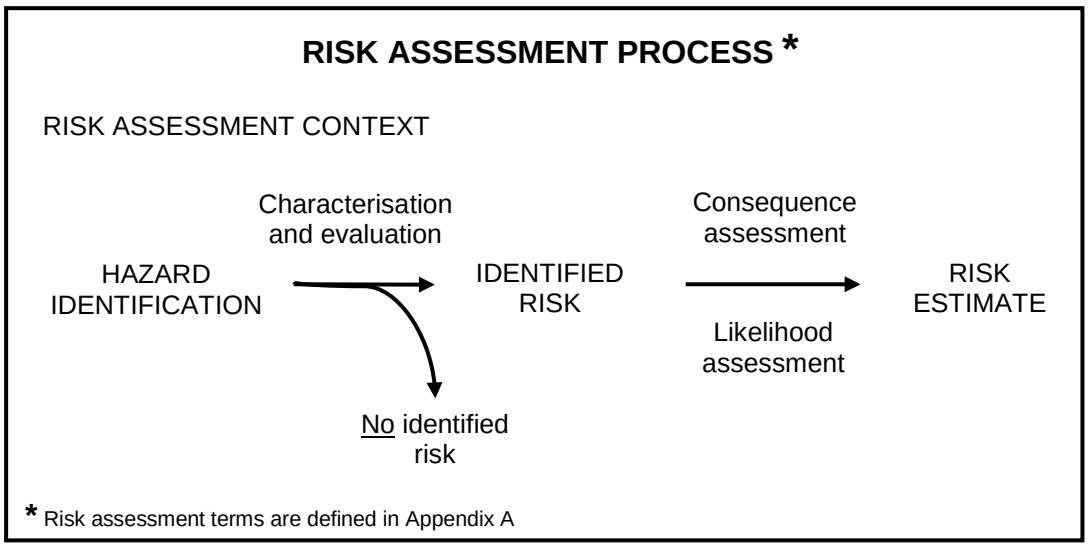
111. Currently there are 4 notifications for field trials of GM *B. napus* in the European Union (http://gmoinfo.jrc.it/). Since 1991 there have been 372 such notifications for GM oilseed rape (*B. napus* as well as other closely related species) in the European Union.

Chapter 2 Risk assessment

Section 1 Introduction

112. Risk assessment is the overall process of identifying the sources of potential harm (hazards) and determining both the seriousness and the likelihood of any adverse outcome that may arise. The risk assessment (summarised in Figure 3) considers risks from the proposed dealings with the GMOs that could result in harm to the health and safety of people or the environment posed by or as a result of gene technology.

Figure 3 The risk assessment process



113. Once the risk assessment context has been established (see Chapter 1) the next step is hazard identification to examine what harm could arise and how it could happen during a release of these GMOs into the environment.

114. It is important to note that the word 'hazard' is used in a technical rather than a colloquial sense in this document. The hazard is a source of potential harm. There is no implication that the hazard will necessarily lead to harm. A hazard can be an event, a substance or an organism (OGTR 2005).

115. Hazard identification involves consideration of events (including causal pathways) that may lead to harm. These events are particular sets of circumstances that might occur through interactions between the GMOs and the receiving environment as a result of the proposed dealings.

116. A number of hazard identification techniques are used by the Regulator and staff of the OGTR, including the use of checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2005). In conjunction with these techniques, hazards identified from previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

117. The hazard identification process results in the compilation of a list of events. Some of these events lead to more than one adverse outcome and each adverse outcome can result from more than one event.

Section 2 Hazard characterisation

118. The list of events compiled during hazard identification are characterised and evaluated to determine which events represent a risk to the health and safety of people or the environment posed by or as a result of gene technology.

119. A risk is identified only when there is some chance that harm will occur. Those events that do not lead to an adverse outcome or could not reasonably occur do not represent an identified risk and will not advance in the risk assessment process. Risks associated with the remaining events are assessed further to determine the seriousness of harm (consequence) and chance of harm (likelihood). The identified risks must be posed by or result from gene technology.

120. The criteria used by the Regulator to determine harm are described in Chapter 3 of the *Risk Analysis Framework*. Harm is assessed in comparison to the parent organism and in the context of the proposed dealings and the receiving environment. The risk assessment process focuses on measurable criteria for determining harm.

121. The following factors are taken into account during the analysis of events that may give rise to harm:

- the proposed dealings, which may include experimentation, development, production, breeding, propagation, and possession, use, supply, transport or disposal of the GMOs during the course of these dealings
- the size, duration and locations of the release
- containment measures proposed by the applicant
- comparisons with the non-GM parents
- 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
- properties of the biotic and abiotic environment at the site of release
- agronomic management practices for the GMOs.

122. Events considered during the risk assessment for this application that are discussed in detail later in this Section are summarised below in Table 4. Events that share a number of common features have been grouped together in broader hazard categories. Nineteen events were characterised, none of which were considered to lead to an identified risk that required further assessment.

Table 4 Summary of events that may give rise to adverse outcomes

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.1 Production of a substance toxic to people	1. Ingestion of GM plant materials containing the proteins encoded by the introduced genes.	Toxicity for people	No	• People are exposed to the same or similar proteins from bacteria or plant species through normal diet or the environment. • None of the proteins encoded by the introduced genes have structural similarity to known toxins. • None of the GM plant materials from the proposed release would be used as human food.
	2. Contact with, or inhalation of, GM plant materials containing the proteins encoded by the introduced genes via: • Occupational exposure • Exposure to the wider community.	Toxicity for people	No	• People are exposed to the same or similar proteins from bacteria or plant species through normal diet or the environment. • None of the proteins encoded by the introduced genes have structural similarity to known toxins. • Contact with, or inhalation of GM plant materials would be limited by containment measures proposed by the applicant and be restricted to people working with the GM plants.
Section 2.2 Production of a substance allergenic to people	3. Ingestion of GM plant materials, containing proteins encoded by the introduced genes,.	Allergic reactions in people	No	• People are exposed to the same or similar proteins from bacteria or plant species through normal diet or the environment. • None of the proteins encoded by the introduced genes have structural similarity to known allergens. • None of the GM plant materials from the proposed release would be used as human food.
	4. Contact with, or inhalation of, GM plant materials (including pollen), containing the proteins encoded by the introduced genes via: • Occupational exposure • Exposure to the wider community.	Allergic reactions in people	No	• None of the GM plant materials from the proposed release would be used as human food or the production of oil or for animal feed. • People are exposed to the same or similar proteins from bacteria or plant species through normal diet or the environment. • None of the proteins encoded by the introduced genes have structural similarity to known allergens. • Contact with, or inhalation of GM plant materials would be limited by containment measures proposed by the applicant and be restricted to people working with the GM plants.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.3 Production of a substance toxic to organisms other than people	5. Direct or indirect ingestion of GM plant materials containing proteins encoded by the introduced genes by vertebrates, invertebrates or micro-organisms.	Toxicity for vertebrates, invertebrates or micro-organisms	No	• Vertebrates, invertebrates and micro-organisms are exposed to the same or similar proteins via contact with common bacteria and plant species. • Exposure of organisms to the introduced proteins is expected to be restricted by the limited scale and duration of the proposed release and proposed containment measures, including the use of cages and tents at some release sites. • None of the proteins encoded by the introduced genes have structural similarity to known toxins. • None of the GM plant materials from the proposed release would be used as animal feed.
Section 2.4 Spread and persistence of the GM canola and Indian mustard in the environment	6. Expression of the proteins encoded by introduced genes increasing the spread and persistence of the GM canola and Indian mustard	Weediness	No	• The introduced herbicide tolerance genes would only confer a selective advantage to the GM plants in areas where the specific herbicide for which they have tolerances are applied. • Hybrid vigour is not a function of the genetic modification and GM hybrids would unlikely to be any weedier than non-GM derived hybrids. • Spread and persistence of GM and non-GM canola and Indian mustard are limited by abiotic factors such as water and nutrient availability • The applicant has proposed containment and management measures to limit the spread and persistence of the GM canola and Indian mustard including: ➢ Harvest of GM canola or Indian mustard by hand or direct heading to limit dispersal of seed. ➢ After harvest, monitoring the site for volunteer canola and Indian mustard plants and destroying any volunteers found for 3 years. ➢ After harvest encouraging germination of any residual seed using light tillage.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	7. Dispersal of GM seed or other GM plant materials during transport, research, storage, equipment use, adverse weather or via animals	Weediness	No	• The release sites are not prone to flooding, excess water is likely to pool rather than run off. If flooding did occur, seed would not likely be dispersed beyond the 50m monitoring zone. • Prolonged flooding would render the seed unviable. • Canola and Indian mustard plants do not tolerate water logging. • Plant material within windrows are normally trapped by the remaining stubble and not easily dispersed by wind. • Domesticated animals will not be allowed to graze the GM crops or stubble after harvest. Birds may feed on the ripening seed pods but are unlikely to disperse viable seed. • The applicant has proposed a number of measures to limit the spread and persistence of the GM plants including ➢ Harvest and thresh seed on site ➢ Clean equipment on site ➢ After harvest, clean the site (including the monitoring zone), destroy all plant material not required for further research, and monitor for volunteer canola and Indian mustard plants and destroy any volunteers. ➢ Transport and store seed according to OGTR guidelines.
	8. Increased exposure of vertebrates (including people), invertebrates and micro-organisms to GM canola and Indian mustard volunteers expressing the introduced genes.	Toxicity or allergenicity in people. Toxicity for other vertebrates, invertebrates and/or micro-organisms	No	• The potential effects on people are discussed in Events 1-4. • The potential effects on organisms other than people are discussed in Event 5. • The limited size and duration of the release and containment measures proposed by the applicant including monitoring and destruction of volunteers would limit the spread and persistence of the GM canola and Indian mustard lines.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.5 Vertical transfer of genes or genetic elements to sexually compatible plants	9. Expression of the introduced genes in other *B. napus* or *B. juncea* plants.	Weediness	No	• Outcrossing to any sexually compatible plant would be mainly limited to plants in close proximity which are flowering synchronously. • Outcrossing from GM canola or Indian mustard to non-GM canola and Indian mustard would result in plants containing the same introduced genes. • The spread and persistence of these plants would be limited by the same factors discussed in events 6 and 7 above. • Canola and Indian mustard are not major weeds of natural ecosystems in Australia and are considered to be a poor competitors and do not establish well in undisturbed areas • The trial would be of limited size and duration and the applicant has proposed a number of measures to limit gene flow including: ➢ the use of insect-proof bags, cages and tents to maximise seed purity and limit pollen flow ➢ surrounding the site with a 50m monitoring zone which is free of flowering, sexually compatible plants ➢ the use of pollen traps and isolation zones between the GMOs and the nearest planting of Brassica crop species
	10. Expression of the introduced genes in hybrids between *B. napus* and *B. juncea* plants	Weediness	No	• Hybrids between *B. napus* and *B. juncea* can occur naturally at low frequencies between plants in close proximity. • Hybrid vigour is not a function of the genetic modification and GM hybrids would not be any weedier than non-GM hybrids • GM *B. napus* x *B. juncea* hybrids would only have a selective advantage in areas where the specific herbicides for which they have tolerance are applied. • The applicant has proposed a number of measures to limit gene flow (see event 9)

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	11. Expression of the introduced genes in other Brassica species	Weediness	No	• Naturally occurring hybrids between *B. juncea* and any other Brassica species (except *B. napus*) have not been reported. • Naturally occurring hybrids between *B. napus* and any other Brassica species (except *B. juncea*) have not been reported. • GM *B. napus* and volunteer *B. rapa* are unlikely to be in close proximity because the applicant has proposed surrounding the site with a 50m monitoring zone which would be free of flowering, sexually compatible species. • Interspecific hybrids between *B. napus* and *B. rapa* would be unlikely to spread and persist because they have reduced fertility and seed set. • The applicant has proposed measures to limit gene flow from the GMOs to any sexually compatible species (see event 9)
	12. Expression of the introduced genes in other sexually compatible species	Weediness	No	• Hybridisation may occur at very low frequencies between *B. napus* and some species in the *Brassiceae* tribe. However, the interspecific hybrids are predominantly or partially sterile and there has been no reported evidence of introgression of genes from *B. napus* into these species. • There have been no reported hybrids occurring under natural conditions between *B. juncea* and any species in the *Brassiceae* tribe • Natural hybrids between *B. napus* and other weed species in the *Brassiceae* tribe have not been reported. • There have been no reports of hybrids between *B. napus* or *B. juncea* and other weed species in tribes other than *Brassiceae.* • The applicant has proposed measures to limit gene flow from the GMOs to any sexually compatible species (see event 9)

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
	13. Stacking of herbicide tolerance genes in *B. napus* and *B. juncea* plants.	Weediness	No	• The applicant has proposed measures to limit gene flow from the GMOs to any sexually compatible species (see event 9). • Canola and Indian mustard are not considered major weeds of natural ecosystems in Australia. Plants with multiple herbicide tolerance would not be more weedy or invasive than single herbicide tolerant or non-herbicide tolerant types in natural ecosystems • Canola and Indian mustard with multiple herbicide tolerances and would be unlikely to have an impact on agricultural practices except the choice of herbicides for weed management. • Management of canola or Indian mustard volunteers with multiple herbicide tolerances can be achieved by the application of alternative herbicides and established principles and practices for minimising the development of herbicide resistance in any agricultural weed.
	14. Increased exposure of vertebrates (including people), invertebrates and micro-organisms to other sexually compatible species (including *B. napus* and *B. juncea*) expressing the introduced genes.	Toxicity or allergenicity in people. Toxicity for other vertebrates, invertebrates and/or micro-organisms.	No	• The potential for the introduced genes to increase the spread and persistence of GM canola and Indian mustard or other sexually compatible species was assessed (Events 6-13) and no risk was identified. • The potential for these proteins causing toxic or allergic reactions in people was assessed (Events 1-4) and no risk was identified. • None of the proteins encoded by the introduced genes has significant homology to known toxins or allergens, thus it is unlikely that expression of these genes in species that are sexually compatible with *B. napus* or *B. juncea* would result in increased toxicity or allergenicity.
	15. Presence of the introduced regulatory sequences in sexually compatible species including other *B. napus* and *B. juncea* plants as a result of gene transfer.	Toxicity or allergenicity in people. Toxicity for other vertebrates, invertebrates and/or micro-organisms. Weediness	No	• The introduced regulatory sequences operate in the same manner as regulatory elements endogenous to canola and Indian mustard plants. The transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects. The impacts from the introduced regulatory elements are equivalent and no greater than the endogenous regulatory elements.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.6 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	16. Presence of the introduced genes or regulatory sequences in other organisms as a result of gene transfer.	Toxicity or allergenicity in people. Toxicity for other vertebrates, invertebrates and/or micro-organisms. Weediness Increased pathogenicity	No	• The introduced genes or similar genes and the introduced regulatory sequences are already present in the environment and are available for transfer via demonstrated natural mechanisms. • Gene transfer from plants to bacteria has not been demonstrated under natural conditions, and the likelihood of such transfer is greatly exceeded by the likelihood of transfer from other natural sources of this gene.
Section 2.7 Unintended changes in biochemistry, physiology or ecology	17. Altered levels of innate toxic or allergenic compounds as a result of the genetic modification.	Toxicity or allergenicity in people. Toxicity for other vertebrates, invertebrates and/or micro-organisms. Weediness	No	• Compositional analysis of the GM canola and Indian mustard lines proposed for release has not been done as the proposed trial represents early stage research. • Compositional analysis of similar GM InVigor® canola lines containing the same *barnase* and *barstar* genes showed no anti-nutritional effects or significant compositional differences compared with non-GM canola. • The above studies suggest that the *barnase* and *barstar* genes did have not a pleiotropic effect on endogenous genes related to toxicity or allergenicity and would be unlikely to have an effect in the GM lines proposed for release. • No obvious altered phenotypes resulting from the expression of the introduced genes have been observed in overseas glasshouse or field trials of the GMOs proposed for release. • The limited scale and duration of the proposed release, along with containment measures proposed by the applicant (see event 9), will limit any possible adverse outcomes
	18. Altered biochemistry, physiology or ecology of the GM canola and Indian mustard lines resulting from the genetic modification.	Toxicity or allergenicity in people. Toxicity for other vertebrates, invertebrates and/or micro-organisms. Weediness	No	• Unintended adverse effects, if any, would be limited by the scale and duration of the proposed release as well as containment measures proposed by the applicant (see event 9). • Results from overseas glasshouse and field trials show no evidence of altered biochemistry, physiology or ecology of the GM canola and Indian mustard lines compared to the non-GM parent. • Off types would likely be identified and discarded during the evaluation of agronomic performance. • None of GM plant materials or products from the GM plants will be used as human food or animal feed.
Section 2.8 Unauthorised activities	19. Use of GMOs outside the proposed licence conditions (non-compliance)	Potential adverse outcomes mentioned in Events 1-18	No	• 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.

2.1 Production of a substance toxic to people

123. Toxicity is the cascade of reactions resulting from exposure to a dose of a chemical that is sufficient to cause direct cellular or tissue injury, or otherwise inhibit normal physiological processes (Felsot 2000). Toxic proteins are known to act via acute mechanisms rather than through chronic exposure (Sjoblad et al. 1992). Toxicity may occur through ingestion, contact or inhalation. The level of toxicity is often expressed as the LD_{50}. This is the amount of a substance given in a single dose that causes death in 50% of a test population of an organism.

124. Toxicity assays generally use the purified toxin of interest rather than the product that expresses the protein (eg GM plant material). This is necessary because the aim of the assays is to determine the concentration of toxin at which an adverse effect is seen. The level of expression in the product is used to determine the level of exposure to the toxin and comparison to the results of the toxicity assay indicate whether or not this is a safe level of exposure (OECD 1998; Konig et al. 2004). The use of purified toxin also increases the reproducibility of the assays.

Event 1: Ingestion of GM plant materials containing the proteins encoded by the introduced genes

125. Exposure to the proteins encoded by the introduced genes could occur as a result of consumption of material from the GM canola and Indian mustard lines. Traditionally, people consume Indian mustard (*B. juncea*) as a leafy vegetable and seeds are used to prepare the condiment (mustard) or essential oils. Canola oil, with low levels of two naturally occurring toxicants, erucic acid and glucosinolates, is a common food ingredient (OGTR 2002).

126. The toxicity of the proteins encoded by the introduced genes was considered previously in Section 4.2 of Chapter 1. The BARNASE and BARSTAR proteins are not known to be toxic to people. Oil from GM InVigor® canola lines expressing both the BARNASE and BARSTAR proteins has been previously assessed and approved for commercial release in Australia and overseas (see Section 6, Chapter 1). Similarly, as outlined in Section 4.2 of Chapter 1, the introduced genes responsible for herbicide tolerance traits do not encode known toxic proteins.

127. The *barstar* and *barnase* genes are derived from *B. amyloliquefaciens*. The *barnase* gene encodes an RNAase and the *barstar* gene encodes its inhibitor. RNAases and RNAase inhibitors are ubiquitous in nature and serve many biological functions. Additionally, *B. amyloliquefaciens* is a commonly occurring soil bacterium and is frequently used as a source for industrial enzymes such as α-amylase. The proteins produced by the introduced genes that confer herbicide tolerance, or similar proteins (see Section 4.2.2, Chapter 1), are found in various bacteria and plant species and therefore present in the environment. Thus, people are already exposed to the same or similar proteins through normal diet or the environment.

128. The applicant does not intend to use GM plant materials from the proposed release in human food or as animal feed, but to destroy all plant materials other than some materials collected for research or seed for future planting or shipping overseas. Ingestion of materials from the GM canola and Indian mustard lines containing the introduced proteins is not expected to occur from the proposed release.

129. Therefore, **no risk is identified**, and the potential for toxicity for people as a result of ingestion of the proteins encoded by the introduced genes will not be assessed further.

Event 2: Contact with, or inhalation of, GM plant materials containing the proteins encoded by the introduced genes

130. Exposure to the proteins encoded by the introduced genes could occur as a result of contact with, or inhalation of, material from the GM canola and Indian mustard lines. This may occur via occupational exposure or general exposure to the wider community from living near the proposed release site.

131. Exposure of people to the proteins encoded by the introduced genes may occur via dermal and inhalation contact when the plant cells have been damaged or broken or via pollen. People working with damaged GM canola or Indian mustard plants may come into contact with the introduced proteins during handling and/or processing the GM lines or their products that contain the proteins. Exposure to BARNASE or BARSTAR proteins is expected to be very limited because they are not expressed in the mature pollen due to tissue specific promoters with limit expression to tapetum cells during anther development.

132. Bayer has proposed a limited and controlled release, with containment measures (Section 2.3, Chapter 1) to limit the spread and persistence of the GM canola and Indian mustard. These measures include a 50m monitoring zone and isolation zones of 400 or 1000m. Canola pollen distribution determined from pollen counts shows a steep decline with distance and most canola pollen travels less than 10 m (Salisbury 2002b). A similar pollen distribution pattern is expected for Indian mustard. Therefore, people living in close proximity to the release site would have limited exposure to GM pollen. In addition, some of the GM lines are male sterile and bagging, cages and tents will be used at some sites which will further limit pollen dispersal and exposure of people. Furthermore, people are already exposed to the same or similar proteins through normal diet or the environment.

133. The applicant does not intend to use GM plant materials from the proposed release in human food or as animal feed, but to destroy all plant materials other than some materials collected for research or seed for future planting and/or shipping overseas. This would further limit dermal contact with or inhalation of materials from the GM canola and Indian mustard lines containing the introduced proteins.

134. Dermal and inhalation toxicity studies have not been conducted with the proteins encoded by the introduced genes. However, on the basis of the presence of these or similar proteins in bacteria and plant species and no known toxic effects of exposure, they are expected to be of very low acute dermal and inhalation toxicity.

135. Therefore, **no risk is identified** and the potential for toxicity for people as a result of contact with, or inhalation of, GM plant materials containing the proteins encoded by the introduced genes will not be assessed further.

2.2 Production of a substance allergenic to people

136. The possibility that exposure of people to the introduced proteins expressed by the GM canola and Indian mustard plants may result in an allergic reaction is considered. Routes of exposure to the introduced proteins could include consumption of food containing canola and Indian mustard products or dermal contact with material from GM plants such as oil, canola or mustard seed meal or pollen as a result of occupational exposure or inhalation of pollen.

Event 3: Ingestion of GM plant material, containing protein encoded by the introduced genes.

137. None of the introduced proteins are known to cause allergic reactions nor do they have significant homology with any proteins known to cause allergenic reactions (refer section 4.2,

Chapter 1). Oil from GM InVigor® canola lines expressing both the BARNASE and BARSTAR proteins have been previously assessed and approved for commercial release and human consumption in Australia and overseas (see Section 6, Chapter 1). People are already exposed to the same or similar proteins through normal diet or the environment.

138. Typically, oil extracted from canola and Indian mustard for human consumption does not contain proteins and the meal remaining after oil extraction is not normally consumed by people, but is used for animal feed. None of the GM canola and Indian mustard materials from the proposed release would be used in human food or animal feed. Thus, the potential for allergic reactions in people resulting from exposure to food is unlikely.

139. Therefore, **no risk is identified** and the potential for production of a substance allergenic to people will not be assessed further.

Event 4: Contact with, or inhalation of, GM plant materials (including pollen), containing the proteins encoded by the introduced genes

140. Exposure to the proteins encoded by the introduced genes could occur as a result of contact with, or inhalation of, material from the GM canola and Indian mustard lines. This may occur via occupational exposure or general exposure to the wider community from living near the proposed release site. Routes of exposure to these proteins would be the same as those outlined in event 2 (above). People's contact with or inhalation of materials from the GM canola and Indian mustard lines containing the introduced proteins is expected to be very limited as a result of the proposed release.

141. None of the introduced proteins are known to cause allergic reactions nor do they have significant homology with any proteins known to cause allergenic reactions (refer section 4.2, Chapter 1). Oil from GM InVigor® canola lines expressing both the BARNASE and BARSTAR proteins has been previously assessed and approved for commercial release and human consumption in Australia and overseas (see Section 6, Chapter 1). People are already exposed to the same or similar proteins through normal diet or the environment.

142. Therefore, **no risk is identified** and the potential for allergic reactions in people resulting from contact with GM plant materials (including pollen) containing the proteins encoded by the introduced genes will not be assessed further.

2.3 Production of a substance toxic to organisms other than people

143. A range of organisms may be exposed directly or indirectly to the proteins encoded by the introduced genes in the GM canola and Indian mustard. Organisms may be exposed directly to these proteins through biotic interactions with GM plants, root exudates or dead plant material, or through contact with vertebrates, insects, symbiotic micro-organisms and/or pathogenic fungi. Indirect exposure could occur in organisms that feed on organisms that feed on GM canola and Indian mustard or degrade them (vertebrates, insects, fungi, Oomycetes and/or bacteria).

Event 5: Direct or indirect ingestion of GM plant materials containing the proteins encoded by the introduced genes by vertebrates, invertebrates or micro-organisms

144. Vertebrates such as livestock and wildlife may be exposed to the GM canola and Indian mustard lines containing the proteins encoded by the introduced genes through direct feeding or indirectly through consumption of other organisms which have fed on the GM plants.

145. Invertebrates could be exposed to the GM canola and Indian mustard and to the proteins encoded by the introduced genes through feeding on the plants, seeds or pollen, or via the soil when the GM plant tissue decomposes. Exposure of soil invertebrates to these

proteins could occur as a result of contact with root exudate that may contain the introduced proteins. Exposure could also occur indirectly through consumption of other organisms that have fed on the GM plants.

146. The applicant proposes to deploy bees or flies when cages and/or tents are used during the trial to facilitate pollination. The bees and flies would be introduced into cages and either die naturally or be destroyed using insecticides prior to removal of the cages. Bees and hives would be kept within enclosed tents from prior to flowering until podding occurs (seed formation) and then moved to a non-Brassica crop.

147. Bees as a primary pollinator would have the greatest exposure to the proteins encoded by the introduced genes. Based on the tissue specific expression of the BARNASE and BARSTAR proteins in GM InVigor® canola (refer to RARMP for DIR 021/2002), it appears unlikely that these proteins will be expressed in the pollen of the GM canola and Indian mustard lines proposed for release. However, as the trial is early stage research to examine agronomic performance, expression levels in specific plant tissues for all the proteins encoded by the introduced genes have not yet been determined.

148. Relative exposure would be greatest for herbivorous species (such as locusts) feeding on the GM plants. Sap feeders (such as aphids) would have minimal exposure to proteins as the sap is primarily composed of sugars and mineral salts dissolved in water. Insects such as ants could remove seeds or plant material for consumption and be exposed through this method.

149. Micro-organisms, particularly soil micro-organisms, will be exposed to the GM canola and Indian mustard plants and the proteins encoded by the introduced genes during the growth and decomposition of plant material. After harvest of the GM plants the remaining canola and Indian mustard residues will be destroyed or buried in the soil so that soil micro-organisms are likely to be exposed to the proteins as the residues are broken down. The level of exposure is likely to decrease with time, as a result of protein degradation in the soil.

150. As indicated in Section 4.2 of Chapter 1, the *barnase* and *barstar* genes are derived from a common soil bacterium, *B. amyloliquifaciens,* and thus soil micro-organisms are likely to have been exposed naturally to the proteins encoded by these genes in the environment. Similarly, the same or similar proteins for herbicide tolerance are present in bacteria and plant species and therefore vertebrates, invertebrates and micro-organisms would be exposed naturally to these proteins in the environment. It is considered that the GM canola and Indian mustard would be no more toxic than non-GM canola and Indian mustard (see Sections 2.1 above).

151. Contact with, or ingestion by vertebrates, invertebrates or micro-organisms of the GM plant materials containing the proteins encoded by the introduced genes would be restricted by the limited scale and duration of the proposed release, the use of cages and tents at some of the release sites and none of the materials would be used as animal feed.

152. Therefore, **no risk is identified** and the potential for toxicity for organisms (other than people) as a result of direct or indirect indigestion of the proteins encoded by the introduced genes will not be assessed further.

2.4 Spread and persistence of the GM canola and Indian mustard lines in the environment

153. Information on non-GM canola and Indian mustard is included here to establish a baseline for comparison with the GMOs being considered in this risk assessment. Attributes of non-GM canola and Indian mustard associated with weediness are discussed in Section 3 of

Chapter 1 and further details about canola are in the document *The Biology and Ecology of Canola* (Brassica napus) (OGTR 2002). The weed status of canola and Indian mustard have been considered previously in the RARMPs prepared for licence application DIR 010/2001, 021/2002, 032/2002, and 057/2004. These documents conclude that non-GM canola and Indian mustard are not serious weeds in Australia.

154. As discussed in Chapter 1, Section 3.1.2, Indian mustard and canola have a number of 'weedy traits'. Indian mustard is reported to be more tolerant to heat and moisture stress than canola which could potentially result in increased persistence. It is however, less prone to pod shattering than canola. Indian mustard has the same weediness traits of induced seed dormancy, self- and cross-pollination and unspecialised pollinators as canola (Oram et al. 2005), but also like canola, it is considered to be a poor competitor and does not establish well in undisturbed areas (Oram et al. 2005; CFIA 2005) such as natural ecosystems (Groves et al. 2003). Both canola and Indian mustard establishment are limited by water and nutrient availability, 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.

Event 6: Expression of the proteins encoded by introduced genes increasing the spread and persistence of the GM canola and Indian mustard

Potential increased weediness due to the introduced hybrid breeding system

155. The potential for increased weediness depends on whether or not there are any attributes conferred by the proteins encoded by the introduced genes that would act to increase the likelihood of weediness of the GMOs over that of non-GM canola and Indian mustard plants.

156. The GM canola and Indian mustard lines proposed for release differ from conventional canola and Indian mustard in the expression of proteins conferring herbicide tolerance and regulating the hybrid breeding system (BARNASE and BARSTAR). These traits would not be expected to result in changes to the intrinsic weediness of the GM canola and Indian mustard. Studies of four GM crops (including GM canola) indicated that none of the GM crops examined were more invasive or more persistent than their conventional counterparts (Crawley 1992; Crawley et al. 2001a).

157. The insertion of the hybrid breeding system and the herbicide tolerance genes into the GM canola and Indian mustard has the potential to alter the phenotypic characteristics of the canola and Indian mustard plants. The phenotypic changes could include altered growth habit, seed shedding or out-crossing tendency. However, previous releases of similar GM canola and Indian mustard lines containing genes for herbicide tolerance and the hybrid breeding system (see section 6, Chapter 1) have reported no adverse effects. Any gene disruptions caused by the genetic modification are likely to be detrimental to the canola and Indian mustard plants and would be selected against in the early glasshouse stages of this project, as random gene disruptions are rarely advantageous (Kurland et al. 2003).

158. The primary elements of the hybrid breeding system are the male sterility and fertility restoration lines. The potential for the introduced genes to provide a selective advantage has been assessed previously in the RARMPs prepared for licence applications DIR 010/2001, 021/2002, 032/2002, and 057/2004. These documents concluded that genes expressing the BARNASE and BARSTAR proteins were unlikely to increase the weediness of canola and Indian mustard for the following reasons:

- the male sterile (MS) plants rely on other pollen to produce seed, thus their ability to persist would be less than that for conventional canola or Indian mustard

- the phenotype of the fertility restoration (RF) lines is similar to that of conventional canola and Indian mustard and is not expected to impact weediness of the GM lines
- the hybrid vigour displayed in the GM hybrid plants is not a function of the genetic modification but is a result of the breeding of the two genetically distinct parents and any selection advantage resulting from hybrid vigour would unlikely be any greater than that for conventionally bred hybrids utilising the non-GM cytoplasmic male sterility system
- male sterility in the GM lines is unlikely to increase the weediness potential any more so than would cytoplasmic male sterility in conventional breeding
- hybrid vigour characteristics are unlikely to confer an advantage outside agricultural fields where resources are limited
- in general, and importantly, hybrid vigour manifested in the F_1 generation declines in subsequent generations

Potential increased weediness due to introduced herbicide tolerance

159. The herbicide tolerance traits of the GM lines provides for a possible selective advantage over conventional non-GM canola and Indian mustard. In situations where a herbicide is applied exclusively for weed control, the GM plants that are tolerant would be able to survive and potentially persist if they reach maturity and set seed.

160. Currently there is no evidence that GM herbicide tolerant crops are more invasive than their conventional counterparts (Crawley et al. 1993; Crawley et al. 2001b). Traits that lead to weediness in plants tend to be polygenic traits and would not be easily conferred by adding individual herbicide tolerance genes.

161. In general terms, it is unlikely that the herbicide tolerance traits would significantly enhance the weediness potential of the GMOs over that of non-GM canola and Indian mustard except when the herbicides to which they are tolerant are applied. The GMOs are susceptible to other herbicides so control could be achieved by their use and/or by non-chemical techniques that are all part of weed management best practice.

162. Bayer proposes management measures for this release that includes the removal and destruction of all canola and Indian mustard plants from the release site for three years after harvest and cleaning of the site.

Persistence of GM canola and Indian mustard

163. Pod shattering can disperse seeds over short distances, but Indian mustard has pods that do not shatter readily. In contrast, canola pods are prone to shattering and seeds may be dispersed during harvest. Bayer has proposed a mixture of hand and machine harvest of seed from the release sites. Hand harvest would reduce spillage and the likelihood of soil seed bank accumulation that can result from machine harvesters. Bayer has indicated that the GM canola would be cut and left in windrows to dry prior to threshing, which creates an opportunity for dispersal through pod shattering and handling. In contrast, the Indian mustard would likely be direct headed (threshed without being cut and dried) which would reduce dispersal of seed.

164. Following harvest of the GM canola and Indian mustard from the trial site, some remaining non-viable plant material would be incorporated into the soil. Additionally, some seed may fall to the ground at maturity and during harvest and may be incorporated into the soil. Both canola and Indian mustard can persist in the agricultural environment, particularly as volunteer plants in subsequent seasons.

165. Neither canola nor Indian mustard seeds exhibit primary dormancy. Thus, at harvest, mature seeds will germinate under appropriate conditions. Seed dormancy (or secondary dormancy) may be induced by environmental factors and buried seed may remain viable in the soil for nearly 3 years or more if measures are not taken to encourage germination. Both canola and Indian mustard are considered to be poor competitors and do not establish well in unmanaged areas (Oram et al. 2005; CFIA 2005).

166. Bayer proposes release of the GM canola and Indian mustard in both winter and summer growing seasons. Analysis of monitoring reports from Australian trial sites of other GM canola indicate that at the majority (82.5%) of winter sown GM canola trial sites, no volunteers were recorded in the third year after harvest (Salisbury 2002a; Salisbury 2002c).

167. While Indian mustard is reported to be more tolerant to heat and moisture stress than canola, Indian mustard plants will still die and seeds will not germinate under significant stress. Germination of GM canola and Indian mustard seed is no different than that of the parent organisms under glasshouse or field conditions (Bayer 2006), which suggests that expression of the introduced genes has not altered seed viability, germination rates and/or seedling viability.

168. To ensure that GM canola and Indian mustard does not persist at the release sites, Bayer proposes to monitor the site for emergence and subsequent destruction of volunteers for three years after the last harvest. Additionally, Bayer has proposed 'light tillage' of the site after harvest so that secondary dormancy due to deep burial is not induced in residual canola and Indian mustard seed. It is anticipated that germination will occur readily under irrigation, which is necessary during the summer season or rainfall during the winter season.

169. Based on the above information, **no risk is identified** and the potential for increased spread and persistence due to expression of the proteins encoded by the introduced genes will not be assessed further.

Event 7: Dispersal of GM seed or other GM plant materials during transport, research, storage, equipment use, adverse weather or via animals

170. In the course of the proposed dealings the applicant proposes to transport seed to and from the release sites, cultivate GM canola and Indian mustard plants, store the GM seed harvested from the crop and collect GM plant materials for research purposes, laboratory research or possible future release (subject to further applications and approvals). The applicant proposes to destroy all plant materials other than some materials collected for future research, planting or shipping of seeds overseas. Accidental spillage or dispersal of GM plant materials, especially seed, in the course of these dealings could allow the GM canola and Indian mustard plants to spread and persist in the environment.

171. Both canola and Indian mustard can establish in disturbed environments, normally as a result of human activities such as seed spillage during transport. However, both are considered to be poor competitors and do not establish well in unmanaged areas (Oram et al. 2005; CFIA 2005). Unless the habitat is regularly disturbed, or seed replenished from outside, canola will be displaced by other plants (Salisbury 2002c) and this is also expected to be true for Indian mustard.

Dispersal by spillage during transport, research, storage or from equipment

172. The Regulator has issued guidelines and policies for the transport, supply and storage of GMOs (*Guidelines for the transport of GMOs, June 2001* and *Policy on transport and supply of GMOs, July 2005*). Bayer proposes to transport and store seed according to OGTR guidelines. Any GM canola or Indian mustard seed not required for future research or

planting will be destroyed. Therefore, any spillage of seed during transport to and from the release sites or while in storage would be rare. Any incident involving spillage of GM seed is expected to be readily controlled through cleaning and monitoring of the site of the spill. In addition, the opportunity for an adverse outcome from any such rare occurrence is further diminished by the need for appropriate environmental conditions for germination, survival and persistence of any few escaped seeds.

173. Furthermore, the applicant proposes to thoroughly clean equipment (eg. boots, clothing, machinery) on site after the GM canola and Indian mustard has been harvested to prevent dispersal of seed to other locations. The site would be monitored for volunteers for three years and any volunteer canola and Indian mustard plants will be destroyed.

Dispersal by adverse weather

a) Strong winds

174. Widespread natural dispersal of canola and Indian mustard seeds and vegetative propagation of cultivated canola and Indian mustard does not generally occur in the field. While pod shattering can disperse seeds over short distance, it is possible that windrows of GM canola and Indian mustard plant material including seed could be blown into adjacent fields outside the release site. Dispersal distance would depend on the wind strength, the amount of trash on the ground and the moisture content of the material. It is reasonable to expect that strong winds may transport seeds and pods of low moisture content within the field, to adjacent fields or outside agricultural areas.

175. Bayer intends to harvest the GM canola and Indian mustard seed either by hand or machine. For hand harvest, plants are hand cut and then tied and hung upside down in bundles to dry. The bundles will be tied to the cage or tent poles. Once uniformly dry, the seeds are then threshed and the seed packaged and transported according to OGTR transport guidelines. Tying bundles of plants and suspending them to dry would limit dispersal of GM seed or plant material if strong winds were to occur.

176. Bayer has indicated that when machine harvested, GM Indian mustard is typically direct headed (thus it is not cut for drying prior to threshing) and this would also limit dispersal of GM seed due to strong winds. The GM canola would be cut and placed in windrows for drying prior to threshing. During 10 years of GM canola and Indian mustard trials in Australia, movement of plant material off the release site due to strong winds has not been observed by the applicant. Winds may move plant material from windrows but this material is caught in the next windrow or trapped by the remaining stubble.

b) Flooding

177. Flooding after planting or at harvest could disperse seed and GM canola and Indian mustard volunteers may establish along waterways (eg drains, creeks and rivers), or in adjacent fields outside the release site. No data exists on seed transport rates by water of canola and other *Brassica* species.

178. In Australia canola and Indian mustard are typically harvested in early autumn and late spring to early summer for summer and winter cultivation, respectively. This corresponds to periods of reduced rainfall when flooding would be less likely to occur. Also, farmers would be unlikely to windrow any crop if heavy or prolonged rainfall were predicted, as this could cause sprouting or disease which would reduce the both the quality and profitability of the crop.

179. The applicant has indicated that sites prone to flooding are avoided as waterlogging is detrimental to good crop establishment. Many of the potential release sites have an option for

irrigation if required and therefore are basically level sites and not prone to run off from application of water. Excess rainfall at these sites would be more likely to result in standing water which would then drain away rather than runoff, which would limit dispersal of GM seed from the release site due to flooding.

180. As seeding of canola is generally into moist soil at depths ranging from 2-3 cm (GRDC 2006, http://www.grdc.com.au/growers/as/canola.htm) to 5 cm (Primary Industries and Resources SA, 2006; http://www.pir.sa.gov.au/byteserve/agriculture/agfactsheets/fieldcrops/ canolalw.pdf), flooding would have to be sufficient to transport considerable amounts of topsoil on basically level land to move canola seed off the release site. Therefore flooding would have to be substantial to transport the planted canola seed beyond the applicant's proposed 50 m monitoring zone which surrounds the site, and into waterways.

181. As the specific gravity of bare seed is slightly higher than that of water any seed displaced by flooding would tend to sink, especially after soaking. As the seed is heavier than water, and would be transported along with bed load sediment in rivers and creeks. There is a high likelihood that the majority of seed being transported by water would be carried to positions unfavourable for establishment (OGTR 2002).

182. Prolonged exposure to water would likely render *B. napus* and *B. juncea* seed unviable. Under flooding or waterlogged conditions, there would not be sufficient oxygen present for cell respiration to provide energy for germination to proceed (that is for emergence of the radicle from the seed). In poor germinating conditions, such as waterlogging, the seed is more susceptible to decay from soil micro-organisms (Canola Council Canada, 2006; http://www.canola-council.org/gs_stage1.aspx). Lack of oxygen reduces root respiration, growth and nutrient uptake and can result in plant death (Canola Council of Canada, 2006; http://www.canola-council.org/stress_moisture.aspx).

183. If flooding was not prolonged and displaced seed did not become waterlogged, as canola and Indian mustard seed have no dormancy they would likely germinate. However, without continued irrigation or rainfall, the seedlings would be unlikely to persist. If seedlings established in adjacent fields normal agricultural practices (herbicide application, cultivation) would likely destroy any volunteers. Seedlings established in non-agricultural areas would not likely spread and persist, as canola and Indian mustard are poor competitors and do not establish well in unmanaged areas (Oram et al. 2005; CFIA 2005). Unless the habitat is regularly disturbed, or seed replenished from outside, canola will be displaced by other plants (Salisbury 2002c) and this is expected to be true for Indian mustard.

Dispersal by animals or insects

184. There is also the possibility of seed dispersal occurring due to the activities of animals which may be able to access the sites. The GM canola and Indian mustard proposed for release would not be used for animal feed nor will stock be permitted to graze on GM plants, plant materials or stubble. However, there could be incidental grazing by wildlife in the field, either while growing or as stubble before it is destroyed. It is conceivable that small amounts of seed could disperse in the faeces of animals. In the absence of information available on Indian mustard, it is appropriate to use data on its close relative canola.

185. 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.

186. It is unlikely that dissemination of GM canola and Indian mustard seed by wild birds consuming seed directly from the crop would occur at a significant level. Birds such as cockatoos and sparrows can shred or remove pods during development and at maturity (Stanley & Marcroft 1999). Canola and Indian mustard seeds are soft and are highly unlikely to survive passage through the gut of a bird. Should the seed survive passage through the gut, it is unlikely that significant adverse consequences of gene escape through seed would occur given the small number of seeds likely to survive and the current low weediness status of non-GM canola and Indian mustard in Australia. The scale of the proposed release is small and some sites would be covered by cages and/or tents thereby limiting potential access for birds.

187. A variety of insects are likely to feed on the crop, however it is unlikely that most of these would contribute to the dispersal of material from the GM canola and Indian mustard plants beyond the trial sites. It is possible that ants may remove seeds for underground storage but to depths where germination is highly unlikely.

Conclusion

188. Based on the above information, **no risk is identified** and the potential for increased spread and persistence as a result of dispersal of GM canola and Indian mustard seed or other GM plant materials during transport, research, storage, equipment use, adverse weather or via animals will not be assessed further.

Event 8: Increased exposure of vertebrates (including people), invertebrates and micro-organisms to GM canola and Indian mustard volunteers containing the introduced genes

189. The potential for increased spread and persistence of the GM canola and Indian mustard lines in the environment was assessed in Events 6 and 7 and no risk was identified. However, in the unlikely instance of this occurring, spread and persistence of the GM plants in the environment could lead to increased exposure of vertebrates (including people), invertebrates and micro-organisms to GM canola and Indian mustard volunteers containing the proteins encoded by the introduced genes.

190. An adverse outcome could occur if these proteins were toxic or allergenic for people. The potential for the proteins encoded by the introduced genes causing toxic or allergic reactions in people was assessed in Events 1-4 of this Chapter and no risk was identified.

191. Organisms other than people may be exposed directly, through feeding on the GM plants or indirectly through eating organisms that have fed on or degrade the GM plants as a result of spread and persistence of the GM canola and Indian mustard in the environment. These organisms include vertebrates, invertebrates and micro-organisms. The potential for toxicity of the proteins encoded by the introduced genes to organisms other than people was considered in Event 5 of this Chapter and no risk was identified.

192. The release would be of limited size and duration and the applicant proposes a number of measures to limit the spread and persistence of the GM canola and Indian mustard lines in the environment (for details see Section 2.3 of Chapter 1).

193. Therefore, no risk is identified and the potential for toxicity or allergic reactions in people or other organisms as a result of spread and persistence of the GM canola and Indian mustard lines in the environment will not be assessed further.

2.5 Vertical transfer of genes or genetic elements to sexually compatible plants

194. Transfer of genetic material to offspring by sexual reproduction (vertical gene transfer) could result in the transfer of the introduced genes and associated regulatory

elements to other plants. These plants would include other *B. napus* and *B. juncea* (planted on site, volunteer or commercially grown), other Brassica species, and other sexually compatible species. Vertical gene transfer to other *B. napus* or *B. juncea* could result in GM *B. napus* and *B. juncea* hybrids and stacking of herbicide tolerance among GM and non-GM herbicide tolerant canola.

195. Weediness resulting from an increase in the spread and persistence of other canola or Indian mustard plants is contingent on both of the following steps:

- transfer of the introduced gene(s) to other plants
- weediness of the recipient plants as a result of introgression and expression of the introduced gene(s)

196. The proposed release of the GM canola and Indian mustard lines would take place in areas of commercial canola and Indian mustard production. Thus, there is potential for gene transfer between GM plants proposed for release and non-GM canola and Indian mustard grown on site as controls, volunteers or commercially grown in adjacent fields. Commercially grown canola could include triazine tolerant (TT) and imidazolinone tolerant (Clearfield®) non-GM canola varieties which are currently grown throughout Australia. GM glyphosate tolerant Roundup Ready® and GM glufosinate ammonium tolerant InVigor® canola have been approved for unrestricted release in Australia, but are not grown commercially.

Event 9: Expression of the introduced genes in other B. napus and B. juncea plants

197. GM canola and Indian mustard could outcross with non-GM canola and Indian mustard which are grown as part of the proposed release. They could also outcross to volunteer or commercially grown non-GM canola and Indian mustard grown in adjacent fields. Most canola pollen travels less than 10 m, but on rare occasions it can be transferred up to 1.5 km by bees and up to 4 km by wind. There is limited data on pollen flow for Indian mustard. However, given the similarities between Indian mustard and canola, pollen distribution and outcrossing of Indian mustard is expected to be similar to canola (Salisbury 2006).

198. Overseas studies on canola pollen dispersal have shown a steep decline in rates of outcrossing with distance - outcrossing ranging from 3 to 12% (at 0m), 0.02 to 2.1% (at 47-54m), 0.0038 to 0.6% (at 366 to 400m) (OGTR 2002). Overseas studies have shown outcrossing rates ranging from 0.8 to 5% over distances of 1.2 to 4 km, however, these studies used male sterile (or emasculated plants) which only represent the potential for gene flow, as outcrossing to male fertile plants would likely be much lower (OGTR 2002). None of the commercial canola or Indian mustard lines grown in Australia are known to be male sterile.

199. The only published Australian study did not show this decline in canola pollen dispersal over distance. Instead, variable rates of outcrossing were shown between 0 and 2600 m, with a rate of <0.01% at 3km (Rieger et al 2002). Outcrossing occurred in 63% of the fields sampled but only a few had outcrossing rates greater than 0.03% (Rieger et al. 2002). The 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.

200. Studies of pollen flow in *B. juncea* are somewhat limited. GhoshDastidar et al. (2000) conducted a three year trial in up to 4 locations in India, examining outcrossing from GM Indian mustard to non-GM Indian mustard which surrounded the GM Indian mustard at 5m

intervals. Outcrossing was limited to 35m from the GM Indian mustard pollen source, with no outcrossing occurring between 35 and 50m (the upper limit examined). Maximum outcrossing occurred at 5m (0.244%) and ranged from 0.007 to 0.012% at 35m. Bees were introduced to the trial, allowing for insect and wind mediated transfer of pollen.

201. The applicant proposes a range of containment measures including surrounding release sites with a 50m monitoring zone which would be free of any flowering sexually compatible species, the use of pollen traps, isolation zones between sites and any commercial Brassica crops and, in some instances, physical barriers (such as bags, cages and tents), will further limit gene flow. Additionally, trial sites and 50m monitoring zones will be monitored for volunteer canola and Indian mustard plants for 3 years after harvest of the GMOs and any volunteers found will be destroyed. If volunteer canola and Indian mustard plants are present in an isolation zone, their density would be low, which would also limit gene flow.

202. On the basis of the above information, it is likely that outcrossing (gene transfer) from the GM canola and Indian mustard proposed for release into other canola or Indian mustard planted on site, volunteers, and commercial crops may occur at low frequencies, but proposed containment measures would further limit gene flow.

203. Although outcrossing is likely to occur among plants grown on site in close proximity, the result would be *B. napus* and *B. juncea* with the same introduced traits as the GM lines proposed for release. All seed not required for further research would be harvested and destroyed. Seed intended for future planting will most likely be derived from plants covered with bags, cages or tents which maximise seed purity by limiting unintended outcrossing. Open-pollinated seed are unlikely to be used for future planting because the seed would be of uncertain parentage. Further, the potential for increased spread and persistence of *B. napus* and *B. juncea* due to expression of the introduced genes has been assessed (Events 6 and 7) and no risk was identified**.**

204. Therefore, **no risk is identified** and the potential for expression of the introduced genes in other *B. napus* and *B. juncea* plants leading to increased weediness will not be assessed further.

Event 10: Expression of the introduced genes in hybrids between GM B. napus and B. juncea

205. 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. 1996b). The reciprocal cross was less successful (Jorgensen et al. 1996a; Jorgensen et al. 1996b). *B. napus x B. juncea* hybrids have reduced pollen fertility (ranging from 0 to 28%) but have been shown to produce viable seed and survive to the next generation (Bing et al. 1991; Jorgensen et al. 1996b)

206. GM *B. napus x B. juncea* hybrids may have increased hybrid vigour and expression of the introduced genes could result in increased weediness. However, GM *B. napus x B. juncea* hybrids would not show any more hybrid vigour than non-GM *B. napus x B. juncea* hybrids and expression of the introduced genes would only have a selective advantage in areas where the herbicide for which they have tolerance is applied. Interspecific hybrids would have no additional introduced traits compared to the intraspecific GM hybrid canola and Indian mustard lines proposed for release. The potential for increased spread and persistence of the GM canola and Indian mustard lines in the environment was assessed in Events 6 and 7 and no risk was identified.

207. Hybrids between GM *B. napus* and *B. juncea* would likely occur at the release site where both species would be in close proximity but this would be at a reduced frequency

compared to outcrossing between *B. napus* plants or between *B. juncea* plants which are in close proximity. As discussed in Event 9, it is expected that open-pollinated seed from the site will be destroyed due to uncertain parentage.

208. The applicant has proposed surrounding sites with a 50m monitoring zone which would be free of any flowering sexually compatible species, which would further limit hybridisation between GM *B. napus* and *B. juncea*. The sites and 50m monitoring zones would be monitored for volunteer canola and Indian mustard plants for 3 years after harvest of the GMOs and any volunteers found destroyed, which would limit the spread and persistence of hybrids between GM *B. napus* and *B. juncea*, should they occur. Additionally, the applicant has proposed other containment measures (pollen traps, isolation zones) which would further limit outcrossing.

209. Based on the above information, **no risk is identified** and the potential for expression of the introduced genes in hybrids between the GM canola and Indian mustard plants leading to increased weediness will not be assessed further.

Event 11: Expression of the introduced genes in other Brassica species

210. Of the many Brassica species in Australia, *B. napus* may potentially hybridise *with B. rapa* under natural conditions. *B. rapa* is known as canola, turnip rape and White turnip and the species includes vegetable forms such as turnip, Chinese cabbage, and pak choi. Naturally occurring hybrids between *B. napus* and *B. rapa* have been reported in several overseas countries with spontaneous backcrossing and gene introgression reported to occur at low frequencies. However, naturally occurring hybrids between *B. napus* and *B. rapa* have not been reported in Australia except putatively in plant breeders nurseries where the plants were in close proximity (Salisbury 2006). Naturally occurring hybrids between Indian mustard and *B. rapa* or any other Brassica species (except *B. napus*) have not been reported (Salisbury 2006).

211. Interspecific hybrids between *B. napus* and *B. rapa* have reduced fertility and low seed set. *B. rapa* has previously been grown in Australia as an oilseed crop but is no longer grown commercially in Australia and is not a widespread agricultural weed. The vegetable forms of *B. rapa* are not recognised as weeds in agricultural environments of Australia and they are generally harvested prior to flowering, unless they are grown for seed production (Salisbury 2006). The applicant has proposed containment measures such that release sites (the area planted to GMO and the 50m monitoring zone) would be free of flowering, sexually compatible species (such as *B. rapa*). Thus, hybrids between *B. napus* and *B. rapa* are unlikely to occur because they would not likely be in close proximity. If outcrossing did occur between *B. napus* and *B. rapa*, the hybrids would be unlikely to spread and persist due to reduced fertility. Expression of the introduced genes would only confer a selective advantage in areas where the herbicide for which they have tolerance is applied.

212. Based on the above information, **no risk is identified** and the potential for expression of the introduced genes in hybrids between the GM canola or Indian mustard and other Brassica species leading to increased weediness will not be assessed further.

Event 12: Expression of the introduced genes in other sexually compatible species

213. Naturally occurring hybrids between *B. napus* and sexually compatible species have been reported only for a few species in the tribe *Brassiceae*. Hybrids between *B. napus* and *Raphanus raphanistrum* (wild radish), *Hirschfeldia incana* (Buchan weed) and *Sinapis arvensis* (charlock) have been reported at very low frequencies (approximately 10^{-4} to 10^{-8}) (Salisbury 2002, OGTR BE 2002). Hybridisation followed by backcrossing could lead to

introgression of the introduced genes into the compatible species and potentially to increased weediness of these species.

214. 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 (Salisbury 2002). In addition, after 5 generations of backcrossing, the herbicide tolerance trait had not been introgressed into the *R. raphanistrum* or *H. incana* genomes.

215. Hybrids between *B. napus* and *S. arvensis* have been detected at extremely low frequencies and only if *S. arvensis* is the male parent and *B. napus* is male sterile. All hybrids were sterile. Naturally occurring hybrids between *S. arvensis* and *B. napus* have not been reported when both parents are fully fertile. There have been no reported natural hybrids occurring between *B. juncea* and these three weed species (Salisbury 2002, 2006).

216. Natural hybrids between *B. napus* or *B. juncea* and other weed species in the *Brassiceae* tribe 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* or *B. juncea* and other weed species in tribes other than *Brassiceae* (Salisbury 2002, 2006).

217. If hybrids between *B. napus* and *R. raphanistrum*, *H. incana* or *S. arvensis* were to occur, expression of the introduced genes would only confer a selective advantage in areas where the herbicide for which they have tolerance is applied.

218. Based on the information above, **no risk is identified,** and the potential for expression of the introduced genes in hybrids between the GM canola or Indian mustard and other Brassica species leading to increased weediness will not be assessed further.

Event 13: Stacking of herbicide tolerance genes in B. napus and B. juncea

219. Outcrossing could occur among the GM herbicide tolerant canola and Indian mustard proposed for release and GM InVigor® canola which would be grown on site as a control for the field trials, commercial plantings of non-GM triazine tolerant and imidazolinone tolerant canola varieties. Although they are not currently grown commercially, this event also considers the potential for outcrossing with GM glyphosate tolerant Roundup Ready® and GM glufosinate-ammonium tolerant InVigor® canola varieties, which have been approved by the Regulator for unrestricted release. This outcrossing would result in "stacking" of herbicide tolerance genes such that either canola or Indian mustard may have tolerance to multiple herbicides.

220. During 2005-6, non-GM herbicide-tolerant canola varieties comprised approximately 90-95% of Western Australia's canola crop, with most of this being triazine tolerant varieties. In eastern Australia (SA, Vic and NSW) approximately 85-85% was herbicide-tolerant, with 60-70% triazine tolerant and 15% imidazolinone tolerant varieties (Trent Potter personal communication, 2006). Based on this information, it is highly likely that non-GM herbicide tolerant canola could be planted near the proposed release sites.

221. Non-GM canola and GM canola approved for release in Australia collectively have tolerance to four herbicides (imidazoline, triazine, glyphosate, and glufosinate-ammonium) which belong to 4 different herbicide classes (B, C, M and N, respectively). The GM canola and Indian mustard lines proposed for release have tolerance to herbicide that falls in the same classes as the non-GM herbicide tolerant or previously approved GM canola that may be grown in Australia.

222. Stacking of GM and non-GM herbicide tolerance genes in canola and Indian mustard has previously been considered in the RARMPs prepared for licence applications DIR 010/2001, 021/2002, 032/2002 and 057/2004, and these documents are available on the OGTR website at <http://www.ogtr.gov.au/>. In summary, these documents considered the following information:

- canola volunteers with multiple tolerances were identified in Canada and France
- the frequency of herbicide tolerance stacking between adjoining glyphosate and glufosinate ammonium-tolerant crops was greatest at the interface between the crops (~1%), but within the crop was 0.2 % or less for distances between 50m and 800m from the edge (data from Canada)
- stacking together of glyphosate and glufosinate ammonium tolerance genes into canola did not alter susceptibility to other, unrelated herbicides
- canola and Indian mustard are not considered major weeds of natural ecosystems and plants with multiple herbicide tolerance would not be more weedy or invasive than single herbicide tolerant or non-herbicide tolerant types in natural ecosystems
- canola and Indian mustard with multiple herbicide tolerances would be unlikely to have an impact on agricultural practices except for the choice of herbicides for weed management
- multiple herbicide tolerant canola and Indian mustard volunteers can be controlled by phenoxy herbicides like 2,4-D
- management of canola or Indian mustard volunteers with multiple herbicide tolerances can be achieved by the application of established principles and practices for minimising the development of herbicide resistance in any agricultural weed, including:
  - *informed selection and rotation of herbicides and crops*
  - *attention to the control of volunteers*
  - *maintenance of hygiene in seeding*
  - *careful harvesting and transport operations, and*
  - *implementation of good agronomic practices*

223. The above documents concluded that stacking of herbicide tolerance genes was unlikely to confer any selective advantage compared to non-GM canola and Indian mustard except in agricultural areas where these herbicides are used to control the spread and persistence of canola and Indian mustard. Additionally, although stacking was likely to occur at low frequencies between crops in close proximity, the resulting plants with multiple herbicide tolerances could be managed in an agricultural setting.

224. Bayer has proposed that all sites would be surrounded by a 50m monitoring zone which would be kept free of *Brassica* crop species and any sexually compatible weed species. Bayer has also proposed that sites consisting of all male sterile plants or where all plants are bagged or covered with cages or tents prior to flowering would have a 400m isolation zone between the trial and the nearest Brassica crop. For sites which are not all planted to male sterile plants or bagged/caged/tented, Bayer has proposed surrounding the site with either a 15m pollen trap of male sterile or non-GM canola and Indian mustard and a 400 m or 1000 m isolation zone.

225. Seed resulting from outcrossing between the GM plants proposed for release and the non-GM canola and Indian mustard could be dispersed on site during harvest and persist in subsequent years. The applicant has proposed to monitor the release site for 3 years after harvest of the GMOs and destroy any canola and Indian mustard plants found, which would further limit vertical gene flow.

226. These proposed containment measures would serve to limit outcrossing and therefore limit the stacking of herbicide tolerance genes and/or spread and persistence of volunteers with multiple herbicide tolerances

227. Based on the information above, **no risk is identified,** and the potential for stacking of multiple herbicide tolerance genes in canola or Indian mustard leading to increased weediness will not be assessed further.

Event 14: Increased exposure of vertebrates (including people), invertebrates and micro-organisms to other sexually compatible species (including B. napus and B. juncea) expressing the introduced genes.

228. The introduced genes could be transferred to sexually compatible plants including non-GM volunteer and commercially grown canola and Indian mustard plants, resulting in increased exposure of vertebrates (including people), invertebrates and micro-organisms to the proteins encoded by the introduced genes.

229. The potential for the introduced genes to increase the spread and persistence of GM canola and Indian mustard or other sexually compatible species was assessed (Events 6 to 13 of this Chapter) and no risk was identified. Even if there was an increase in spread and persistence of the GMOs or other sexually compatible species, an adverse outcome could only occur if expression of the proteins encoded by the introduced genes were toxic or allergenic for people.

230. The potential for these proteins causing toxic or allergic reactions in people was assessed (Events 1-4 of this Chapter) and no risk was identified. None of the proteins encoded by the introduced genes has significant homology to known toxins or allergens, thus it is unlikely that expression of these genes in species that are sexually compatible with *B. napus* or *B. juncea* will result in increased toxicity or allergenicity.

231. Therefore, **no risk is identified** and the potential for toxicity or allergenicity to vertebrates (including people), or toxicity to invertebrates and micro-organisms through increased exposure as a result of expression of the introduced genes sexually compatible species (including *B. napus* and *B. juncea*) will not be assessed further.

Event 15: Presence of the introduced regulatory sequences in sexually compatible species including other B. napus and B. juncea plants as a result of gene transfer

232. All of the introduced regulatory sequences operate in the same manner as regulatory elements endogenous to canola and Indian mustard plants. The transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects. The impacts from the introduced regulatory elements are equivalent and no greater than the endogenous regulatory elements.

233. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of vertical gene transfer of introduced regulatory sequences will not be assessed further.

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

Event 16: Presence of the introduced genes or regulatory sequences in other organisms as a result of gene transfer

234. Transfer of the introduced genes, or the introduced regulatory sequences, from the GM canola or Indian mustard plants to sexually incompatible plants, animals or micro-organisms (horizontal gene transfer) rarely occurs without human intervention.

235. Most gene transfers have been identified through analyses of gene sequences (Worobey & Holmes 1999; Ochman et al. 2000). In general, gene transfers are detected over evolutionary time scales of millions of years (Lawrence 1999). Most gene transfers have been from virus to virus (Lai 1992), or between bacteria (Ochman et al. 2000). In contrast, transfers of plant genetic materials to other micro-organisms such as bacteria, viruses or fungi have been exceedingly rare.

236. Transfer of the regulatory sequences to other organisms including viruses could alter the expression of endogenous genes in unpredictable ways. However, all of the introduced regulatory sequences operate in the same manner as regulatory elements endogenous to canola and Indian mustard plants. The transfer of either endogenous or introduced regulatory sequences could result in adverse unpredictable effects. As there is no difference between those two events, this does not represent a novel adverse outcome as a result of the genetic modification.

237. It is possible that recombination could occur between the introduced viral or bacterial promoters and a virus or bacterium invading the GM canola and Indian mustard lines. This could lead to the development of a variant with altered properties. However, these promoter sequences are present in viruses and bacteria which exist naturally in the environment and transfer of sequences from virus to virus, or bacterium to bacterium, is more likely than from plant to virus or bacterium.

238. Horizontal gene transfer has been examined in detail in a number of other RARMPs (most recently DIR 057/2004), which are available from the OGTR website (http://www.ogtr.gov.au) or by contacting the Office. These assessments have concluded that horizontal gene transfer from plants to other sexually incompatible organisms occurs rarely and usually only on evolutionary timescales. Reports of horizontal gene transfer from plants to bacteria occurring during laboratory experiments have not only relied on the use of highly similar sequences to allow homologous recombination to occur, but also on conditions designed to enhance the selective advantage of gene transfer events (Nielsen et al. 2000; Gebhard & Smalla 1998; Mercer et al. 1999; Nielsen 1998; De Vries et al. 2001). Horizontal gene transfer is not expected to produce any adverse outcomes during this proposed limited release.

239. Therefore, **no risk is identified**. The potential for an adverse outcome as a result of horizontal gene transfer will not be further assessed.

2.7 Unintended changes in biochemistry, physiology or ecology

240. A single plant gene can have an influence on multiple, sometimes unrelated, plant traits. This phenomenon is known as pleiotropy. Single genes inserted into a plant by genetic modification can also result in pleiotropy. It is therefore necessary to evaluate GM plants for unintended pleiotropic effects, such as changes in agronomic characteristics, which may be a consequence of the gene insertion.

241. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropic effects (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. Therefore, unintended changes in phenotype, as well as mutations, can occur upon transformation that are similar to those in conventional breeding and mutation breeding (Bradford et al. 2005; Cellini et al. 2004). Recent results using proteomics have indicated that, in potatoes, there are fewer changes between GM and non-GM potatoes than between different conventionally bred varieties (Catchpole et al. 2005; Lehesranta et al. 2005).

242. Possible 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 encoded protein of the introduced gene changing chromatin structure, affecting methylation patterns or regulating signal transduction and transcription
- increased metabolic burden associated with high level expression of the introduced genes
- novel traits arising from interactions of an introduced gene product with 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.

243. Unintended pleiotropic effects might result in adverse outcomes such as toxicity or allergenicity; weediness, pest or disease burden; or reduced nutritional value as compared to the parent organism. However, accumulated experience with genetic modification of plants indicates that 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). Additionally, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003).

Event 17: Altered levels of innate toxic or allergenic compounds as a result of the genetic modification

244. Canola and Indian mustard seed naturally contain the toxicants erucic acid and glucosinolate. However, the term canola refers to those varieties of *B. napus* that meet specific standards for low levels of erucic acid and glucosinolates and produce oils that are safe for human consumption. The requirement for these same low levels applies to *B. juncea* oil (refer to Section 3.1.1 of Chapter 1). There is potential for the GM canola and Indian mustard plants proposed for release to have increased levels of toxic or allergenic compounds as a result of the expression of the introduced genes for herbicide tolerance or hybrid breeding system. Additionally, random insertion of the gene construct during the genetic modification process could potentially result in increased toxic or allergenic compounds in the GM lines.

245. The applicant stated that any GM canola plants which deviated phenotypically from parent lines would have been discarded during early stages of development and selection after genetic modification and during several generations of backcrossing to develop the GM Indian mustard lines. Except for herbicide tolerance and male sterility, no obvious altered phenotypes resulting from the expression of the introduced genes have been observed in overseas glasshouse or field trials. Further phenotypic observations will be made during the proposed release by comparing the agronomic performance of the GM lines relative to non-GM canola and Indian mustard lines and GM InVigor® canola under field conditions.

246. Thus far, exposure to plant materials (including pollen) from the GM canola and Indian mustard lines proposed for release has been limited to a few workers maintaining these plants in the glasshouse or in limited and controlled releases (both occurring overseas). The applicant has reported no adverse outcomes from exposure to the GM canola or Indian mustard plant materials, suggesting that expression of the introduced genes has not altered the toxicity or allergenicity endogenous to non-GM canola and Indian mustard.

247. The potential toxicity and allergenicity of the introduced genes was assessed (see Events 1 to 4 above) and no risk was found. The *barnase* and *barstar* genes have previously been approved for unrestricted release in Australia in GM InVigor® canola and it has been grown commercially in North America for many years with no reports of altered toxicity or allergenicity. Feeding and compositional studies on GM InVigor® canola were presented in DIR 021/2002. These studies demonstrate that there are no anti-nutritional effects or significant compositional differences between GM and non-GM canola. Additionally, the levels of the naturally occurring toxicants of canola, erucic acid and glucosinolates, do not vary between GM and non-GM canola. The above studies on GM InVigor® canola suggest that the *barnase* and *barstar* genes did have not a pleiotropic effect on endogenous genes related to toxicity or allergenicity and would be unlikely to have an effect in the GM lines proposed for release.

248. Data on toxicity of plant material and/or levels of known endogenous toxins would be required for risk assessments of applications for large scale or commercial release of these GM canola and Indian mustard lines. Further information is not required for assessing the risks of this proposed release because exposure to the GMOs would be limited by the proposed size and duration of the release, proposed measures to limit the spread and persistence of the GMOs. Furthermore, none of the GM plant materials or products from the GM plant material are intended for use in human food or animal feed.

249. Therefore, **no risk is identified** and the potential for changes in levels of innate toxic or anti-nutritional compounds as a result of random insertion of the gene construct into the GM canola and Indian mustard lines will not be further assessed.

Event 18: Altered biochemistry, physiology or ecology of the GM canola and Indian mustard lines resulting from the genetic modification

250. Unintended changes in gene expression could alter the biochemistry, the physiology or the ecology of the GM canola and Indian mustard lines. Biochemical, physiological or ecological changes to the GM lines proposed for release could occur either as a result of the expression of the introduced genes or of the transformation process itself. The GM lines proposed for release were selected from a number of initial individual GM events. The applicant stated that any GM canola plants which deviated phenotypically from parent lines would have been discarded during early stages of development and selection after genetic modification and during several generations of backcrossing to develop the GM Indian mustard lines. Based on the applicant's observations, there have been no differences between the parental and GM canola and Indian mustard lines grown overseas under glasshouse or field conditions. Further information is not required at this stage as the proposed release is limited in size and duration and none of the GM plant materials or products from the GM plants are intended for use in human food or animal feed.

251. The potential adverse outcomes (toxicity, allergenicity and weediness) resulting from altered biochemistry, physiology or ecology of the GM canola and Indian mustard lines proposed for release were assessed in Events 1-17 and no risks were identified. GM InVigor® canola containing the same or similar genes has been grown in North America

since 1996 and no adverse outcomes resulting from altered biochemistry, physiology or ecology have been reported (Bayer, 2006).

252. Expression of the introduced genes in the GM canola and Indian mustard lines could lead to altered dormancy, seed viability, germination rate and/or seedling viability resulting in increased spread and persistence of these GM lines. However, there have been no reports of the introduced genes for herbicide tolerance, male sterility and fertility restoration affecting these seed dormancy, viability, and germination or seedling viability. Based on their observations, the applicant has stated that germination of GM canola and Indian mustard seed is no different that that of the parent organisms under glasshouse or field conditions (Bayer 2006), which suggests that expression of the introduced genes has not altered the above seed or seedling traits.

253. The release would be of limited size and duration and the applicant proposes a number of measures to limit the spread and persistence of the GM canola and Indian mustard lines proposed for release (for details see Section 2.3 in Chapter 1).

254. Therefore, **no risk is identified** and the potential for weediness, toxicity or allergenicity to people and other organisms, as a result of unintended changes in biochemistry, physiology or ecology will not be assessed further.

Uncertainty

255. Data on the potential toxicity or allergenicity of the proteins encoded by the introduced genes, and plant materials may be required for risk assessments of applications for larger scale or commercial release of these GM canola and Indian mustard lines. Further information is not required for assessing the risks of this release because the trial is limited in locations, size and duration and none of the GM plant materials are intended for use in human food, animal feed or in the production of other products.

2.8 Unauthorised activities

Event 19: Use of GMOs outside the proposed licence conditions (non-compliance)

256. 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 and Indian mustard lines outside of the proposed release areas. The adverse outcomes that this event could cause are discussed in the Events 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.

257. Therefore, **no risk is identified** and the potential for an adverse outcome as a result of unauthorised activities will not be assessed further.

Section 3 Risk estimate process for identified risks

258. The hazard identification process considered the circumstances by which people or the environment may be exposed to the GMOs, GM plant materials, GM plant by-products, the introduced genes, or products of the introduced genes.

259. Nineteen events were identified and assessed whereby the proposed release of the GM canola and Indian mustard lines might give rise to harm to people or the environment.

260. These 19 events included consideration of whether expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms, produce unintended changes in the biochemistry, physiology or ecology of the GM lines, or alter

characteristics that may impact on spread and persistence of the GMOs. In addition, consideration was given to the opportunity for gene flow to other organisms and its effects if this were to occur.

261. None of the 19 events are considered to give rise to an identified risk that required further assessment. The principal reasons include:

- the scale of the trial that is limited in both area and duration
- containment, monitoring and disposal measures proposed by the applicant to limit the spread and persistence of GM canola and Indian mustard plants
- none of the GM plant materials or products from the GM plants 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 allergenicity from these proteins
- limited capacity of the GM canola and Indian mustard lines to spread and persist in natural ecosystems or undisturbed areas
- limited ability and opportunity for the GM canola and Indian lines to transfer the introduced genes to other sexually related species or other organisms.

262. Therefore, as no risks were identified to the health and safety of people, or the environment, from the proposed release of the GM canola and Indian lines into the environment, the level of risk is considered to be **negligible.**

Chapter 3 Risk management

263. Risk management includes evaluation of risks identified in Chapter 2 to determine whether or not specific treatments are required to mitigate harm to human health and safety, or the environment, that may arise from the release. Other risk management considerations required under the Act are also addressed in this chapter. Together, these risk management measures are used to inform the decision-making process and determine licence conditions that are imposed by the Regulator under the Act. In addition, the roles and responsibilities of other regulators under Australia's integrated regulatory framework for gene technology are also explained

Section 1 Background

264. 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. All licences are required to be subject to three conditions prescribed in the Act.

265. Section 63 requires that each licence holder inform relevant people of their obligations under the licence. Other mandatory statutory conditions contemplate the Regulator maintaining oversight of licensed dealings. For example section 64 requires the licence holder to provide access to premises to OGTR monitors, 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.

266. It is a further requirement that the licence be subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings and the possession, supply, use, transport or disposal of the GMO for the purposes of, or in the course of, a dealing. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Other Australian regulators

267. 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), National Health and Medical Research Council (NHMRC) and Australian Quarantine and Inspection Service (AQIS). Dealings conducted under any licence issued by the Regulator may also be subject to regulation by one or more of these agencies[18].

268. The *Gene Technology Act 2000* requires the Regulator to consult these agencies during the assessment of DIR 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.

[18] 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/pdf/public/raffinal2.2.pdf>.

269. FSANZ is responsible for human food safety assessment, including GM food. As the trial involves research to select lines for possible further development and the applicant does not intend any material from the GM canola and Indian mustard lines to be used for human food. Accordingly, the applicant has not applied to FSANZ for evaluation of materials from the trial for use in human food. FSANZ approval would need to be obtained before such materials could be used for this purpose.

270. The APVMA has responsibility for setting registration conditions for the use of herbicides in Australia, including implementation of herbicide resistance management programs. The canola and Indian mustard lines have been genetically modified for herbicide tolerance. Bayer states that the extent of herbicide application to herbicide tolerant GM lines would not exceed 5 ha nationally per annum. Hence it would be authorised under the APVMA's general, small scale trial permit (APVMA Permit 7250).

271. AQIS is responsible for monitoring imports to prevent the introduction of exotic pests, weeds and diseases into the environment. An importer is required to notify AQIS if they are importing GMOs. Additionally, as the importation of the GM canola and Indian mustard seed constituted a dealing under the *Gene Technology Act 2000*, the importer required an authorisation under this Act for the import to lawfully proceed. Bayer has indicated it intends to obtain a permit from AQIS to import seed of the GM canola and Indian mustard lines.

272. Approval may also be required from the State and Territory Governments that have introduced legislation to delay the commercial introduction of GM canola due to concerns regarding possible market impacts.

Section 3 Risk treatment measures for identified risks

273. The risk assessment of events listed in Chapter 2 concluded that there are **negligible** risks to people and the environment from the proposed release. The *Risk Analysis Framework*, which guides the risk assessment and risk management process, defines **negligible** risks as insubstantial with no present need to invoke actions for their mitigation

274. These events were considered in the context of the proposed release on a maximum of area of 252 hectares on up to 42 sites of no more than 6 ha during 6 growing seasons (summer and winter) between 2007 and 2010, containment measures proposed by the applicant, and the receiving environment (Sections 2 and 5 of Chapter 1).

Section 4 General risk management

275. Containment measures consistent with the risk assessment context have been imposed to limit the trial to size, duration and locations requested by the applicant, which are summarised below.

4.1 Summary of proposed licence conditions

4.1.1 Measures to limit and control the proposed release

276. A number of licence conditions have been imposed to limit and control the release, including requirements to:

- surround the site with a 50m monitoring zone from which related weed species and crop plants would be removed prior to flowering, as well as one of the following measures:
  - *maintain a 1km isolation zone between the site and any other Brassica crop,* ***or***
  - *surround the site with a 15m pollen trap (non-GM canola or Indian mustard) and 400m isolation zone from any other Brassica crop,* ***or***

- *surround the site with a 400m isolation zone from any other Brassica crop if the GMOs at the site are all GM male sterile canola or Indian mustard,* ***or***
- *surround the site with a 400m isolation zone from any other Brassica crop if the all GMOs at the site are covered with cages, tents or selfing bags*

- place cages, tents or selfing bags[19] on flower stalks or over plants at least 7 days prior to flowering until podding (seed formation) of the GM crop
- harvest and store canola and Indian mustard seed from the release separately from commercial Brassica crops
- not permit canola or Indian mustard seed or other materials from the release to be used in human food or animal feed
- treat all pollen trap plants as if they are GM plants
- destroy all GM plant materials not required for further analysis by methods approved by the Regulator
- following harvest, clean each site, monitoring zone and equipment to remove all GM plant materials
- following cleaning, encourage germination of GM seed through the use of 'light' tillage of each site
- monitor for, and destroy any, volunteer GM *B. napus* and *B. juncea* that may occur at each site for 24 months after harvest and thereafter until the site is free of volunteers for a continuous 12 month period.

4.1.2 Measures to control other activities associated with the release

277. The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs, June 2001; Policy on transport and supply of GMOs, July 2005*). Licence conditions based on these guidelines and policies have been imposed regarding transportation and storage, and to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

4.2 Other risk management considerations

278. All DIR licences issued by the Regulator contain a number of general conditions that relate to risk management. These include, for example:

- applicant suitability
- contingency and compliance plans
- identification of the persons or classes of persons covered by the licence
- reporting structures, including a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to the health and safety of people or the environment
- monitoring for compliance.

[19] The use of insect proof cages, tents or selfing bags is proposed to maximise seed purity but when used will also reduce pollen flow and therefore function to limit the spread and persistence of the GMOs.

4.2.1 Applicant suitability

279. 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.

280. Before making the decision to issue a licence for this application (DIR 069/2006), the Regulator considered the suitability of Bayer CropScience Pty Ltd (Bayer) to hold a licence.

281. Conditions in the licence include 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.

282. In addition, any applicant organisation must have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

4.2.2 Compliance and contingency plans

283. The licence requires Bayer to submit a plan detailing how it intended to ensure compliance with the licence conditions and document that compliance. This plan would be required before the planting of the GM canola and Indian mustard lines commences.

284. Bayer is also 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 or Indian mustard lines outside of the permitted areas.

285. Bayer 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 instrument would be 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

286. The persons covered by this 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 this licence.

4.2.4 Reporting structures

287. 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 release
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the release.

288. 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.

289. A number of written notices are required under the licence that will assist the OGTR in designing and implementing its risk based monitoring program for all licensed dealings. The notices would include:

- expected and actual dates of planting
- expected and actual dates of commencement of flowering
- expected and actual dates of final destroying and cleaning at the end of the trial

4.2.5 Monitoring for compliance

290. 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.

291. 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.

292. In cases of non-compliance with licence conditions, the Regulator may also instigate an investigation to determine the nature and extent of non-compliance. These include the provision 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

293. The risk assessment identified additional information that may be required if the applicant were to submit an application for a larger scale trial, reduced containment conditions or a commercial release of any of these GM canola and Indian mustard lines. This would include:

- molecular characterisation of the introduced genetic materials, genotypic stability, and expression levels of the introduced genes in the GM canola and Indian mustard lines
- data on the potential toxicity of plant material from the GM canola and Indian mustard lines including levels of known endogenous toxins
- data on the viability of Indian mustard pollen
- the level of pollen mediated gene flow between both canola and Indian mustard and closely related plants in Australia
- biochemical, physiological and agronomic characteristics of the GM canola and Indian mustard lines indicative of weediness including measurement of germination, seed dormancy, tolerance to environmental stresses (eg heat, drought or disease) and reproductive capacity (eg growth rate and window of flowering) compared to the non-GM parent lines.

Section 6 Conclusions of the RARMP

294. The risk assessment concludes that this proposed limited and controlled release of GM canola and Indian mustard lines on a maximum of 252 hectares on up to 42 sites of no more than 6 ha during 6 growing seasons from 2007 to 2010 in up to 24 shires in NSW, SA and Victoria poses **negligible** risk to the health and safety of people and the environment.

295. The risk management plan concludes that this **negligible** risk does not require specific risk treatment measures. However, licence conditions have been imposed to contain the release to the size, duration and locations requested by the applicant.

Section 7 DIR 069/2006 Licence

296. The licence DIR 069/2006 is available on the OGTR website <http://www.ogtr.gov.au/gmorec/ir.htm#table>, following the path to DIR 069/2006.

297. Information about where the GMOs have been planted pursuant to this licence can be found in a separate document entitled 'DIR 069/2006 Site Details'. This document can be viewed by accessing the OGTR website at http://www.ogtr.gov.au/ir/dir069.htm>.

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Appendix A Definitions of terms in the Risk Analysis Framework used by the Regulator

(* terms defined as in Australia New Zealand Risk Management Standard AS/NZS 4360:2004)

Consequence

outcome or impact of an adverse event

Marginal: there is minimal negative impact

Minor: there is some negative impact

Major: the negative impact is severe

Event*

occurrence of a particular set of circumstances

Hazard*

source of potential harm

Hazard identification

the process of analysing hazards and the events that may give rise to harm

Intermediate

the negative impact is substantial

Likelihood

chance of something happening

Highly unlikely: may occur only in very rare circumstances

Unlikely: could occur in some circumstances

Likely: could occur in many circumstances

Highly likely: is expected to occur in most circumstances

Quality control

to check, audit, review and evaluate the progress of an activity, process or system on an ongoing basis to identify change from the performance level required or expected and opportunities for improvement

Risk

the chance of something happening that will have an undesired impact

Negligible: risk is insubstantial and there is no present need to invoke actions for mitigation

Low: risk is minimal but may invoke actions for mitigation beyond normal practices
Moderate: risk is of marked concern requiring mitigation actions demonstrated to be effective
High: risk is unacceptable unless actions for mitigation are highly feasible and effective

Risk analysis

the overall process of risk assessment, risk management and risk communication

Risk analysis framework

systematic application of legislation, policies, procedures and practices to analyse risks

Risk assessment

the overall process of hazard identification and risk estimation

Risk communication

the culture, processes and structures to communicate and consult with stakeholders about risks

Risk Context

parameters within which risk must be managed, including the scope and boundaries for the risk assessment and risk management process

Risk estimate

a measure of risk in terms of a combination of consequence and likelihood assessments

Risk evaluation

the process of determining risks that require treatment

Risk management

the overall process of risk evaluation, risk treatment and decision making to manage potential adverse impacts

Risk management plan

integrates risk evaluation and risk treatment with the decision making process

Risk treatment*

the process of selection and implementation of measures to reduce risk

Stakeholders*

those people and organisations who may affect, be affected by, or perceive themselves to be affected by a decision, activity or risk

States

includes all State governments, the Australian Capital Territory and the Northern Territory governments

Uncertainty

imperfect ability to assign a character state to a thing or process; a form or source of doubt

Appendix B Summary of issues raised in submissions received from prescribed experts, agencies and authorities[20] on application DIR 069/2006

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 the preparation of the RARMP.

Issues raised relating to the Risk Assessment and where they have been considered:

- Weed status of cultivated Brassica species in and around the proposed release sites (Chapter 1), including longevity of seed in the soil (Chapter 2, Event 6)
- Enhanced spread and persistence (weediness) of the GM canola and Indian mustard lines (Chapters 2, Event 6)
- Toxicity and allergenicity of proteins encoded by introduced genes in the environment (Chapter 2, Events 1-5)
- Dissemination of GM material beyond the intended release area(Chapter 2, Event 7)
- Gene flow to other commercial canola and Indian mustard crops and naturalised populations of cultivated Brassica species (Chapter 2, Events 9-15)
- Hybridisation between GM canola and GM Indian mustard lines (Chapter 2, Event 10)
- Gene flow to sexually compatible weedy species and development of herbicide tolerance in weedy species (Chapters 2, Events 9-15)
- Gene flow to other organisms, including microbes (Chapter 2, Event 16)
- Unintended and potential pleiotropic effects of the introduced genes (Chapter 2, Events 17 and 18).
- Stacking of herbicide tolerance traits in canola, Indian mustard and sexually related species (Chapter 2, Events 9-15)

Issues raised and addressed by the Risk Management Plan (considered in Chapters 3 and 4):

- Containment measures at the field trial sites
- Storage and transport procedures for the GM canola and Indian mustard
- Post-harvest monitoring and practices, such as the effectiveness of shallow cultivation and natural rainfall to encourage germination of residual seed.
- Disposal of GM plant materials not required for further research or approved plantings
- Monitoring of burial site
- Research requirements for future large scale or commercial releases of GM canola and Indian mustard

[20] GTTAC, State and Territory governments, Australian Government agencies, the Minister for Environment and Water Resources ,and Local councils where the release may occur.

Appendix C Summary of issues raised in submissions received from the public on application DIR 069/2006

Two submissions were received from the public. All issues relating to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence.

Issues raised relating to the Risk Assessment and Risk Management Plan and where they have been considered:

- Instability of GMOs (see Chapters 1, Section 4.4 and Chapter 2, Sections 2.7 & 2.8)
- Concern that the size of release is too large and uncontrollable (see Chapters 1, 2, and 4)

Issues that are outside the scope of assessments conducted under the *Gene Technology Act 2000* and corresponding State and Territory laws:

- Increased herbicide usage
- Segregation of GM and non-GM crops

Appendix D Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 069/2006

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

Abbreviations: APVMA: Australian Pesticides and Veterinary Management Authority; **FSANZ**: Food Standards Australia New Zealand; **GM**: genetically modified H: human health and safety; **LC**: licence conditions; **OSA**: outside scope of the assessment

Summary of issues raised	Issue	Consideration of issue
Theoretical or simulated tests using purified proteins are necessary but not sufficient to determine toxicity or allergenicity of GMOs. Actual feeding trials are needed using the GM canola or Indian mustard seed-meal (resulting from extracting the oil) on the animals which it might normally consume the seed-meal as feed. See for example the effect of feeding GM soy mixed with normal rat feed, in which 55.6% of the rat-pups died within 3 weeks of birth compared with 9% of the non-GM soy group (study by Irina Emakova). See also results from CSIRO's examination of GM peas that turned out to have unexpected immune effects in mice.	H	Bayer's purpose in conducting this limited and controlled release is to compare numerous GM canola and Indian mustard lines in order to select those with superior agronomic characteristics for further field trials and possible future release. Feeding trials are not necessary at his stage as no GM plant material is permitted to be used in human food or animal feed (see Section 2, Chapter 1 of the RARMP and licence conditions). The study by referenced has not been published in a peer reviewed journal so the experimental design, methodology, results and statistical analysis can not be independently assessed by other researchers. In contrast, the peer reviewed study by Brake and Evenson (2004) cited in the RARMP showed no differences between mice fed non-GM or GM soybean feed. The use of whole GM foods in feeding studies is considered to be of limited value. Canola seed is not normally consumed by rats and it is difficult to introduce sufficient quantities to provide exposure to the novel proteins as they are expressed at very low levels without creating dietary imbalance that results in adverse nutritional effects that are unrelated to the GMO. The animal testing of CSIRO's GM peas occurred after lines with superior agronomic characteristics were identified, but before any application was made to FSANZ for use in human food. Even though the method used has not been validated to predict harm to humans, the research was subsequently terminated. This example demonstrates that the approach of collecting research data throughout the development process works to effectively identify and prevent potential adverse consequences.
Objection to the release of these GMOs on the grounds of over use of herbicide on herbicide tolerant GE species. Stress (human health factor) involved in avoiding ubiquitous goods, such as mustard and canola, if they are genetically altered and unacceptable to some people.	OSA	The use and safety of herbicides is the responsibility of the APVMA. Labelling of food is the responsibility of FSANZ.