

Risk Assessment and Risk Management Plan

Application for licence for dealings involving an
intentional release into the environment

DIR 021/2002

**Title: Commercial release of
genetically modified (InVigor® hybrid) canola**

Applicant: Bayer CropScience Pty Ltd

25 July 2003



Office of the
Gene Technology Regulator

Abbreviations

AAFC	Agriculture and Agri-Food Canada
ANZFA	Australia New Zealand Food Authority
ALS	acetolactate synthase
APHIS	Animal and Plant Health Inspection Service
APVMA	Australian Pesticides and Veterinary Medicines Authority (formerly NRA)
<i>bar</i>	bialaphos resistance gene
BLAST	Basic Local Alignment Search Tool
CaMV	cauliflower mosaic virus
DEFRA	The Department of Environment, Food and Rural Affairs, UK
DDBJ	DNA Databank of Japan
DIR	dealing involving intentional release
DNA	deoxyribonucleic acid
ELISA	enzyme linked immunosorbent assay
EMBL	European Molecular Biology Laboratory
FAO	Food and Agriculture Organisation of the United Nations
FDA	Food and Drug Administration (USA)
FSANZ	Food Standard Australia New Zealand
g	gram
GM	genetically modified
GMAC	Genetic Manipulation Advisory Committee
GMO	genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
IgE	immunoglobulin E
IOGTR	Interim Office of the Gene Technology Regulator
kD	KiloDaltons
km	Kilometre
m	Metre
MAFF	UK Ministry of Agriculture, Fisheries and Food (now called DEFRA)
mRNA	messenger ribonucleic acid
Ms1, Ms8	Male sterile lines
NOS	nopaline synthase
<i>nptII</i>	neomycin phosphotransferase II
NPTII	neomycin phosphotransferase II enzyme
NRA	National Registration Authority for Agricultural and Veterinary Chemicals (now APVMA)
<i>ocs</i>	octapine synthase gene
OECD	Organisation for Economic Cooperation and Development
OGTR	Office of the Gene Technology Regulator
PAT	phosphinothricin acetyltransferase
PCR	polymerase chain reaction
PDB	The Protein Data Bank
PPT	phosphinothricin (glufosinate ammonium)
ppm	parts per million
PR	planned release

Rf1, Rf2, Rf3	Fertility restorer lines
RNA	ribonucleic acid
Rubisco	ribulose-1,5-bisphosphate carboxylase
Sm/Sp	Gene conferring resistance to aminoglycoside antibiotics streptomycin and spectinomycin
T0, T1, T2, T3	transformed generations
T-DNA	transfer deoxyribonucleic acid
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
WHO	World Health Organisation
µg	Micrograms
µm	Micromoles
mg	Milligrams

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EXECUTIVE SUMMARY

INTRODUCTION

The *Gene Technology Act 2000* (the Act) and the Gene Technology Regulations 2001 (the Regulations) set out requirements which the Gene Technology Regulator (the Regulator) must follow when considering an application for a licence to intentionally release a genetically modified organism (GMO) into the environment.

For a licence to be issued, the Regulator must be satisfied that the release will not pose any risks to human health and safety or the environment that can not be managed. To this end, Section 51 of the Act requires the Regulator to prepare a risk assessment and risk management plan (RARMP) for each licence application, in consultation with a wide range of expert groups and key stakeholders, including the public.

The Regulator has taken into account all matters relevant to the protection of human health and safety and the environment that were raised during the consultation process in finalising the RARMP for application number DIR 021/2002. Information on the submissions received and how they were taken into account is contained in Chapter 2 and Appendix 10.

LICENCE DECISION

On 25 July 2003 the Regulator issued a licence to Bayer CropScience Pty Ltd (Bayer) approving the commercial release of genetically modified (GM) InVigor® hybrid canola, including lines T45, Topas19/2, MS1, RF1, RF2, RF3 and MS8.

THE APPLICATION

Bayer applied for a licence (application number DIR 021/2002) for the commercial release of seven (7) similar GM ‘lines’¹ of canola: T45, Topas19/2, MS1, RF1, RF2, RF3 and MS8. Lines MS1, MS8, RF1, RF2 and RF3, and hybrids derived from MS x RF crosses, are covered by the registered trade name InVigor® canola.

Hybrid seed from the lines RF3 and MS8 would be marketed as InVigor® in Australia. Although Bayer does not intend to commercialise the other five lines in Australia at this time, the applicant sought approval for all seven GM canola lines to achieve consistency with existing overseas regulatory approvals.

Table 1 summarises the modifications that are present in the seven Bayer GM canola lines proposed for release.

Table 1: Genetic modifications in the seven GM canola lines

Line	Glufosinate ammonium tolerance	Hybrid breeding system (InVigor®)	Antibiotic resistance
T45	<i>Pat</i>	–	–
Topas 19/2	<i>Pat</i>	–	<i>npIII</i>
MS1	<i>Bar</i>	<i>barnase</i>	<i>npIII</i>
RF1 and RF2	<i>Bar</i>	<i>barstar</i>	<i>npIII</i>
MS8	<i>Bar</i>	<i>barnase</i>	–
RF3	<i>Bar</i>	<i>barstar</i>	–

¹ The term ‘line’ has been used throughout this risk assessment. ‘Line’ is used to denote canola with a specific genetic modification derived from a single transformation event.

The GM canola from the proposed release would be used as oil in human food, or in animal feed, in the same way as conventional (non-GM) canola.

All seven lines are approved for growing and human consumption in the USA and Canada, and oil derived from all seven canola lines has been approved for use in human food in Australia. (ANZFA 2001a).

The hybrid canola seed which Bayer seeks to commercialise in Australia as InVigor[®] canola is produced with a novel hybrid generation system. This system is based on two genetically modified ‘parent’ lines of canola: a male sterile (MS) line that contains a male sterility gene (*barnase*), and a fertility restorer (RF) line containing a fertility restorer gene (*barstar*).

The development of the pollen-producing parts of canola flowers (anthers) is suppressed in MS plants. Crossing an MS line with an RF line overrides the suppression and makes the progeny fertile. The progeny are expected to have enhanced agronomic performance, otherwise known as ‘hybrid vigour’ (see Appendix 1 for more information).

Naturally occurring male sterile plants are routinely used in conventional (non-GM) breeding systems as a means to control breeding and produce more vigorous plant offspring.

All seven GM canola lines include a gene that confers tolerance to the herbicide glufosinate ammonium. The herbicide tolerance serves as a dominant marker for the introduced traits during breeding and hybrid seed production. It also enables glufosinate ammonium to be used for the control of weeds in the GM canola crop.

The Australian Pesticides and Veterinary Medicines Authority (APVMA), formerly known as the National Registration Authority (NRA), has granted Bayer registration of glufosinate ammonium for use on InVigor[®] canola under the trade name Liberty[®]. The APVMA has registered Liberty[®] for use only InVigor[®] canola crops, not for weed control in other crops. Glufosinate ammonium is not registered for use in any other broad-acre cropping in Australia. However, glufosinate ammonium is also registered as Basta[®] for weed control in horticultural crops and Finale[®] for weed control in non-crop agricultural areas, commercial and industrial areas and rights-of-way. Appendix 4 contains further details.

Four of the GM canola lines contain a gene that provides a ‘marker’ for antibiotic resistance in plants. This gene is used to identify and select modified plants during the development stage. Bayer does not intend to commercialise any of these lines.

Under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC), Bayer (formerly AgrEvo, Aventis CropScience) conducted 14 field trials (PR62, PR63 and extensions) with all seven GM canola lines in Queensland, New South Wales, Victoria, Tasmania, South Australia and Western Australia. In addition, the Regulator issued a licence on 30 July 2002 to Bayer (DIR010/2002) to conduct a limited and controlled release of the same GM canola lines at 30 trial sites, totalling 106 hectares, in New South Wales, Victoria and South Australia for the summer and winter growing seasons in the three years from 2002-03. There have been no reports of adverse effects on human health or the environment resulting from any of these releases.

Some detailed technical information on precise gene constructs and molecular characterisation data included in Bayer’s original application and subsequent material supplied in response to OGTR requests has been declared ‘Confidential Commercial Information’. In accordance with section 184 of the Act this technical information is not available to the general public. However the information was available to the expert groups which are required to be consulted on the preparation of the RARMP.

THE EVALUATION PROCESS

Licence application DIR 021/2002 from Bayer has been evaluated, and a risk assessment and risk management plan (RARMP) prepared, in accordance with the Act and the Regulations, using a Risk Analysis Framework. This framework was developed by the Regulator in consultation with the public and key local, State, Territory and Commonwealth government stakeholders and the Gene Technology Technical Advisory Committee, and is available at www.ogtr.gov.au/pdf/public/raffinal.pdf.

Details of the process that the Regulator must follow, including the prescribed consultation process on the application, and the matters that must be considered in preparing a RARMP, are set out in Appendix 9. The complete, finalised RARMP can be obtained from the OGTR or from the OGTR's web site at www.ogtr.gov.au.

The risk assessment considered information contained in the application (including information required by the Act and the Regulations on the GMO, the parent organism, the proposed dealings and potential impacts on human health and safety and the environment), submissions received during consultation and current scientific knowledge.

As mentioned above, the use of Liberty[®] herbicide (a formulation of glufosinate ammonium) has been registered by the APVMA for use on InVigor[®] canola crops in Australia. As part of the assessment of this use, the APVMA considered potential human health and environmental effects, for example arising through occupational exposure or residues. The APVMA also considered a number of issues that are outside the scope of the Gene Technology Regulator's assessment, such as the efficacy of the herbicide and herbicide resistance management.

Through the risk assessment process, potential hazards to human health and safety or the environment that may be posed by the commercial release of the Bayer canola were identified. These were evaluated on the basis of the likelihood of each hazard occurring and the likely impact of the hazard, were it to be realised. These hazards were considered and evaluated previously for limited and controlled trials with the same GM canola under licence application DIR 010/2001. They were reassessed for this release to determine whether the proposed commercial scale, and the removal of specific licence conditions for containment measures to limit the movement of the GMOs and the introduced genes, posed any additional risks. The identified potential hazards relate to:

- ***toxicity and allergenicity for humans:*** could the GM canola lines be more toxic or allergenic than non-GM canola as a result of the novel gene products or because of unintended effects?
- ***toxicity and allergenicity for other organisms:*** could the GM canola lines be harmful to other organisms including mammals (other than humans), livestock, wildlife, other insects and microorganisms as a result of the novel gene products or because of unintended effects?
- ***weediness:*** could the genetic modifications be harmful to the environment by increasing the potential for the GM canola lines to establish as problem weeds?
- ***transfer of introduced genes to other organisms:*** could there be adverse consequences from possible transfer of the new genes in the GM canola lines to non-GM canola crops, closely related *Brassica* weeds, other brassicaceous weeds, or to other organisms?

- **herbicide resistance:** could weeds develop resistance to herbicide if the InVigor[®]-Liberty[®] crop-herbicide combination is used inappropriately?

Considerations outside the scope of the assessment

There has been considerable speculation in the media and other forums, as well as in some submissions, about the possible impact of the uptake of GM canola on non-GM farmers and upon international export markets.

Feedback from extensive stakeholder consultation during the development of the *Gene Technology Act 2000* made it clear that the community wanted the regulatory system to focus exclusively on the protection of human health and safety and the environment. This is to prevent the possibility of economic considerations such as cost-benefit analyses, market access and agricultural trade implications compromising the regulatory system's focus upon the scientific evaluation of risks and the protection of human health and safety and the environment. As a result, economic and cost-benefit considerations were expressly excluded from the scope of the assessments conducted under the Act.

Therefore, this RARMP does not draw any conclusions about the possible costs or benefits of the Bayer canola to individual farmers, or on market impacts for the agricultural industry.

However, there are a number of industry and government initiatives (independent of this assessment) which do focus on economic and marketability considerations in relation to the adoption of GM canola by the Australian agriculture industry. These include:

- indicative principles of the Commonwealth, State and Territory governments' Plant Industries Committee (circulated as *Guidelines for Industry Stewardship Programs and Crop Management Plans for the Management of Genetically Modified Crops in Australian Farming Systems*)
- the (industry-based) Gene Technology Grains Committee's *Canola Industry Stewardship Protocols for Coexistence of Production Systems and Supply Chains*. The Gene Technology Grains Committee protocols are available from <http://www.avcare.org.au>.
- the Productivity Commission report *Modelling Possible Impacts of GM Crops on Australian Trade* available at <http://www.pc.gov.au/research/staffres/gmcrops/index.html>
- the Australian Bureau of Agricultural & Resource Economics (ABARE) report *Australian Grains Industry 2003-GM Canola. What are its economics under Australian conditions ?* available from <http://www.abareonlineshop.com/product.asp?prodid=12526>

Bayer also submitted a draft version of the *InVigor[®] Canola Crop Management Plan* as part of its application. All of the above documents were analysed in detail for any information of relevance to the assessment. They are summarised in Appendix 7.

CONCLUSIONS OF THE RISK ASSESSMENT

The Regulator considers that the risks to human health and safety, or to the Australian environment, from the commercial release of any of Bayer's seven GM canola lines are no greater than those posed by non-GM canola ie they are as safe as conventional canola. The assessment of each identified potential hazard is summarised under a separate heading below.

Toxicity or allergenicity to humans and other organisms

The GM canola lines are very unlikely to prove more toxic or allergenic to humans or other organisms than conventional canola. Therefore the risks are considered negligible and it is not considered necessary to impose any management conditions in relation to potential toxicity or allergenicity. As noted above, FSANZ has previously approved the use in food of oil from the seven GM canola lines, concluding that products from these GM canolas are as safe as are those from non-GM canola.

Weediness

The risk of the genetic modifications making this GM canola more invasive or persistent than conventional canola in Australia is negligible.

The growth characteristics and agronomic performance of the seven GM canola lines are within the range of conventional canola. The hybrid vigour displayed in InVigor[®] canola hybrids is not a function of the genetic modification, results from the breeding of the two genetically distinct parents. The growth characteristics and agronomic performance of InVigor[®] canola hybrids are within the range of conventional canola hybrids.

The introduced genes do not confer a selective advantage in the absence of the herbicide glufosinate ammonium. Glufosinate ammonium is not registered for use in any broad-acre agriculture except on Bayer's GM InVigor[®] canola. It is used in viticulture and horticulture but is rarely used in non-agricultural areas.

Therefore it is not considered necessary to impose any conditions to manage the risk of weediness.

Transfer of introduced genes to other organisms

The introduced genes do not confer any selective advantage in the absence of the herbicide glufosinate ammonium. The hybrid vigour displayed in InVigor[®] canola hybrids is not a function of the genetic modification that can be transferred as a single trait, but is a result of the breeding of the two genetically distinct parents.

The likelihood of some gene transfer from the GM canola to other cultivated canola is high but diminishes rapidly away from close proximity to the crop, hence the overall frequency of out-crossing will be low. If gene transfer to other canola did occur, as explained above, no competitive environmental advantage is conferred. It remains susceptible to the control measures currently used on conventional (non-GM) canola and can be managed in the same way. Therefore, transfer of introduced genes to other canola crops poses negligible risk and does not require the imposition of specific management conditions.

The likelihood of some transfer of the introduced genes to the closely related weedy *Brassica* species *B. rapa* and *B. juncea* is high, though less than for conventional (non-GM) canola. And, due to the lower incidence of these species and the reduced 'fitness' of any progeny *eg. vigour, fertility etc*, the overall frequency and persistence will be considerably lower. If gene transfer to *B. rapa* or *B. juncea* did occur, it would not confer a selective advantage in the absence of the herbicide glufosinate ammonium. Gene transfer to *B. rapa* poses a very

low risk while the risk posed by gene transfer to *B. juncea* would be negligible. Outcrossing to *B. oleracea* would be unlikely and the risks posed by this would be negligible. Gene transfer to any of these three species would not require any specific management conditions under the *Gene Technology Act 2000*.

The likelihood of transfer of the introduced genes from the GM canola to the less closely related brassicaceous weed species *Raphanus raphanistrum*, *Hirschfeldia incana* and *Sinapis arvensis* is very low, because of genome incompatibility and the severely reduced fitness of any progeny. The overall frequency of outcrossing will be very low. Although these species are weeds of both agricultural and disturbed habitats, they are not considered invasive weeds of undisturbed environments. Even if the glufosinate ammonium tolerance trait was transferred to these species it would not pose any additional risks for the control of these weeds (glufosinate ammonium is known to be ineffective for the control of *R. raphanistrum*). Therefore it is concluded that gene transfer to *R. raphanistrum*, *H. incana* and *S. arvensis* poses a very low risk, and no additional management practices would be needed to control any transgenic hybrids, if they occur, and management strategies would be the same as for other brassicaceous weeds.

The likelihood of gene transfer to any other brassicaceous species is considered negligible. Even if gene transfer to these species did occur, it would not pose any additional risks for the control of these weeds.

The likelihood of transfer of the introduced genes to other organisms is negligible, but even if such transfer did occur it would be unlikely to pose any hazard to human health and safety or to the environment.

Herbicide resistance

There is a potential for development of herbicide-resistant weeds if the InVigor[®] crop-Liberty[®] herbicide combination is used inappropriately. The APVMA has noted that the resistance management plan as contained in Bayer's *InVigor[®] Canola Crop Management Plan* is an essential part of managing herbicide resistance and will be effective in managing the development of resistance to glufosinate ammonium. The APVMA requires that the plan be available to all users of Liberty[®] herbicide. The APVMA has regulatory responsibility and oversight for agricultural chemical use and has stipulated a number of conditions on the use of Liberty[®] herbicide on InVigor[®] canola crops. Therefore no herbicide resistance management conditions are required under the *Gene Technology Act 2000*.

Industry stewardship proposals

The Bayer *InVigor[®] Canola Crop Management Plan*, and industry guidelines developed to assist all participants in the agricultural supply chain achieve coexistence between different production systems e.g. GM/non-GM, GM/organic, were considered in detail in the course of evaluating the application.

The industry stewardship proposals focus on good agricultural and handling practices. The stated aims of the proposals are to:

- enable separation of GM and non-GM crops to the extent required by markets;
- maximise the effective life of the technology; and
- contribute to agricultural sustainability.

The evaluation of this material concluded that there was no information that added to, or impacted on, the risks posed to human health and safety or the environment by the activities

proposed in the application. The risk assessment process evaluated risks that might occur in the *absence* of any supply chain management controls or product stewardship measures.

InVigor[®] hybrid canola will be supplied through accredited resellers from 2004. Growers will be required to sign a grower agreement and will be trained to follow the Crop Management Plan (CMP). The stated aims of the CMP are to ensure awareness of the industry protocols for coexistence of GM and other canola and knowledge of the regulatory conditions placed on the seed and herbicide.

Although it is considered there are no risks from Bayer GM canola that require management to protect human health and safety or the environment, governments and the agricultural industry are still assessing the impact of the commercial release of GM canola on trade and marketability.

THE RISK MANAGEMENT PLAN (KEY LICENCE CONDITIONS)

The Regulator considers that the proposed release does not pose risks to the health and safety of people or the environment in Australia that require management through specific licence conditions (refer to Conclusion of the Risk Assessment, above). Accordingly, the licence the Regulator has issued in respect of the Bayer application DIR 021/2002 contains only minimal oversight conditions. The key licence conditions are outlined below.

Toxicity or allergenicity to humans and other organisms

Based on the risk assessment, no management conditions have been imposed in relation to toxicity or allergenicity.

Weediness

Based on the risk assessment no management conditions have been imposed in relation to weediness.

Transfer of introduced genes to other organisms

Based on the risk assessment no management conditions have been imposed in relation to the transfer of introduced genes to other organisms.

The licence includes a condition that requires the applicant to provide the Regulator with a testing methodology that is able to reliably detect the presence of each of the GMOs or their genetic material.

Herbicide resistance

No conditions have been imposed in relation to the management of herbicide resistance, as this is the responsibility of the APVMA. The licence holder's obligation to comply with any conditions imposed by the APVMA is noted in the licence.

Reporting conditions

Bayer sought regulatory approval for seven GM canola lines, although it has indicated that only lines RF3 and MS8 will be commercialised in Australia as InVigor[®] canola. The licence includes a condition that Bayer report to the Regulator the amount of each GM canola line sold commercially or otherwise grown in each growing season for each State and Territory.

As part of the ongoing commitment to making information publicly available, the Regulator intends to report on the implementation of the InVigor® canola release after three years of commercial plantings. The Regulator has indicated that she will call for public input to the proposed report as part of the responsible oversight of the progress of this and other licences for genetically modified crops.

General conditions

Any licence issued by the Regulator contains a number of general conditions, which are also relevant to risk management. These include, an obligation to inform the Regulator if the applicant becomes aware of any additional information about risks to human health or safety or to the environment.

The licence holder is also obliged to comply with all other relevant Commonwealth, State and Territory legislation.

Monitoring and enforcement of compliance by the OGTR

As well as the legislative capacity to enforce compliance with licence conditions, the Regulator has additional options for risk management. The Regulator can direct a licence holder to take any steps the Regulator deems necessary to protect the health and safety of people or the environment.

In this regard, the reporting requirements imposed by the licence conditions will enable the Regulator to monitor and review the progress of all commercial releases of GM crops in Australia.

FURTHER INFORMATION

Detailed information on the evaluation of the application, including the licence conditions, is available in the risk assessment and risk management plan document for this application, which can be obtained from the website of the Office of the Gene Technology Regulator (www.ogtr.gov.au), or by calling 1800 181 030 (please quote application number DIR 021/2002).

CHAPTER 1 BACKGROUND

1. This chapter provides information about the background to the application and previous releases of relevant GMOs into the environment.

SECTION 1 THE APPLICATION

Project Title:	Commercial release of genetically modified canola (<i>Brassica napus</i>) for use in the Australian cropping system
Applicant:	Bayer CropScience Pty Ltd* 391-393 Tooronga Rd East Hawthorn VIC 3123 *Formerly Aventis CropScience Pty Ltd.
Common name of the parent organism:	Canola
Scientific name of the parent organism:	<i>Brassica napus</i>
Modified traits:	Hybrid breeding system, herbicide tolerance and antibiotic resistance (not in lines proposed for commercial release)
Identity of the genes responsible for the modified traits:	<i>bar</i> gene from the bacterium <i>Streptomyces hygroscopicus</i> in some lines (herbicide tolerance) or <i>pat</i> gene from the bacterium <i>Streptomyces viridichromogenes</i> in some lines (herbicide tolerance) <i>barnase</i> gene from the bacterium <i>Bacillus amyloliquefaciens</i> (male sterility, hybrid breeding system) <i>barstar</i> gene also derived from <i>B. amyloliquefaciens</i> (fertility restorer, hybrid breeding system) <i>nptII</i> gene from the bacterium <i>Escherichia coli</i> in some lines (antibiotic resistance)
Proposed Location	Potentially all canola growing regions of Australia.
Proposed Size of Release:	Small scale first year introduction in south-east Australia, up to full commercial release in all canola growing regions.
Proposed Date of Release:	From Winter 2003
2.	Bayer sought regulatory approval for seven similar genetically modified ‘lines’ ² of canola: T45, Topas 19/2, MS1, RF1, RF2, RF3 and MS8. Lines MS1, MS8, RF1, RF2

² The term ‘line’ has been used throughout this risk assessment. ‘Line’ is used to denote canola with a specific genetic modification derived from a single transformation event.

and RF3 and hybrids derived from MS x RF crosses are covered by the registered trade name InVigor[®] canola.

3. All seven lines are approved for growing and consumption in the USA and Canada. They have all been trialed previously in Australia under limited and controlled conditions and have been approved for use in human food in Australia. (ANZFA 2001a) The lines RF3 and MS8 would be marketed as InVigor[®] in Australia. Although Bayer does not intend to commercialise the other five lines in Australia at this time, the applicant is seeking approval for all seven GM canola lines to achieve consistency with existing overseas regulatory approvals.

Section 1.1 The proposed dealings

4. Bayer sought approval for the commercial release of its GM canola in all canola growing regions of Australia and continued product development and research programs. Proposed areas of the release include all Australian States and Territories.
5. It is intended that Bayer's GM canola plants and their by-products would be used in the same manner as conventional canola. Canola is primarily grown for its seeds, which yield oil and high protein animal feed. Canola oil is used in a variety of products including low-fat foods, pharmaceuticals, margarine, nutritional supplements and salad dressings. During the processing of (GM and non-GM) canola oil, DNA and proteins are removed. The oil derived from all seven lines has been approved by Food Standards Australia New Zealand (FSANZ, formerly the Australia New Zealand Food Authority, ANZFA) for human consumption (ANZFA 2001a).

Section 1.2 Parent organism

6. The parent organism is canola (*Brassica napus*), which is exotic to Australia and is grown as an agricultural crop in New South Wales, Queensland, Victoria, South Australia, Western Australia and Tasmania. More detailed information on canola can be found in a review document 'The Biology and Ecology of Canola (*Brassica napus*)' that was produced in order to inform this risk assessment process. This document is available at the OGTR website (<http://www.ogtr.gov.au>).

Section 1.3 Genetic modification and its effects

7. Five of the seven GM canola lines (RF1, RF2, RF3, MS1 and MS8) have been modified to introduce a novel hybrid breeding system for canola, based on genetically modified male sterile (MS) and fertility restorer (RF) lines. All seven of the GM canola lines have been genetically modified to introduce tolerance to the herbicide glufosinate ammonium. Four of the seven lines have also been modified to introduce an antibiotic resistance marker gene. The genetic modifications introduced into the various GM canola lines are summarised in Table 1.
8. The genetic modifications introduced into the male sterile and fertility restorer lines of InVigor[®] canola enable a breeding system for the production of hybrid canola seed. Hybrid canola varieties produced using conventional (non-GM) breeding techniques have also been developed. Non-GM hybrid canola is estimated to represent about 6% of the Australian canola market. Bayer's GM canola lines also confer resistance to the herbicide glufosinate ammonium. Non-GM triazine and imidazolinone tolerant canola varieties currently comprise approximately 60% of the Australian canola market (Norton 2003).

9. The hybrid canola seed which Bayer seeks to commercialise in Australia as InVigor[®] canola is produced using a novel hybrid generation system. The hybrid generation system is based on two genetically modified ‘parent’ lines of canola: the MS line which contains a male sterility gene (barnase); and the RF line containing a fertility restorer gene (barstar).
10. The development of the pollen producing parts of canola flowers (anthers) is suppressed in MS plants. Crossing an MS line with an RF line overrides the suppression and makes the progeny fertile. The progeny are expected to have enhanced agronomic performance, otherwise known as ‘hybrid vigour’. A more detailed explanation of this ‘hybrid vigour’, which is also utilised in conventional breeding, is provided in Appendix 1)

Table 1: Genetic modifications in Bayer canola lines

GM canola Line	Introduced Genes		
	Glufosinate ammonium tolerance	Hybrid breeding system	Antibiotic resistance
T45	<i>pat</i>	-	-
Topas 19/2	<i>pat</i>	-	<i>nptII</i>
MS1	<i>bar</i>	<i>barnase</i>	<i>nptII</i>
MS8*	<i>bar</i>	<i>barnase</i>	-
RF1	<i>bar</i>	<i>barstar</i>	<i>nptII</i>
RF2	<i>bar</i>	<i>barstar</i>	<i>nptII</i>
RF3*	<i>bar</i>	<i>barstar</i>	-

* Lines proposed to be commercialised in Australia

11. The male sterility gene (barnase) and a fertility restorer gene (barstar) are both derived from *Bacillus amyloliquefaciens*, a common soil bacterium. Both genes are linked to a herbicide tolerance gene: the bar gene. The bar gene, derived from a soil bacterium *Streptomyces hygroscopicus*, codes for the enzyme phosphinothricin acetyl transferase (PAT) which detoxifies phosphinothricin (glufosinate-ammonium), the active ingredient in the herbicides Liberty[®], Basta[®] and Finale[®]. (Liberty[®] has been registered by the APVMA for use in InVigor[®] canola crops).
12. An antibiotic resistance gene (npt II) has been transferred into lines Topas 19/2, MS1, RF1 and RF2. This gene is derived from the bacterium *Escherichia coli* and codes for the enzyme neomycin phosphotransferase, which detoxifies antibiotics such as kanamycin and neomycin, thereby conferring resistance to the bacteria in which the recombinant plasmids are maintained. This is mainly used as a selectable marker for the early selection of transformed plants in tissue culture.
13. Short regulatory sequences that control expression of the genes are also present in the genetically modified canola. These sequences are derived from the cauliflower mosaic virus, *Agrobacterium tumefaciens*, *Arabidopsis thaliana* and *Nicotiana tabacum*. Although the first two organisms are plant pathogens, the regulatory sequences comprise only a small part of their total genome and are not in themselves capable of causing disease.

14. Detailed information on the bar, pat, barnase, barstar and nptII genes, the regulatory sequences, characterisation of the inserted genetic material and the new proteins expressed for the 7 GM canola lines is provided in Appendix 1.

Table 2: Genetic elements and their origin.

Gene	Promoter	Terminator
<i>Bar</i> <i>Streptomyces hygrosopicus</i>	PSsuAra <i>Arabidopsis thaliana</i>	3'g7 <i>Agrobacterium tumefaciens</i>
<i>Pat</i> <i>Streptomyces viridichromogenes</i>	P-35S Cauliflower mosaic virus	T-35S Cauliflower mosaic virus
<i>Barnase</i> <i>Bacillus amyloliquifaciens</i>	PTA29 <i>Nicotiana tabacum</i>	3' -nos <i>Agrobacterium tumefaciens</i>
<i>Barstar</i> <i>Bacillus amyloliquifaciens</i>	PTA29 <i>Nicotiana tabacum</i>	3' -nos <i>Agrobacterium tumefaciens</i>
<i>NptII</i> <i>Tn5 of Escherichia coli</i>	P-nos <i>Agrobacterium tumefaciens</i>	3' -ocs <i>Agrobacterium tumefaciens</i>

Section 1.4 Method of gene transfer

15. The canola lines are generated by inserting the various genes on a plasmid vector carried by *Agrobacterium tumefaciens* (a bacterium). The vector is 'disarmed' since it lacks the genes that encode the tumour-inducing functions of *A. tumefaciens* (see Appendix 1 for details).

SECTION 2 PREVIOUS RELEASES AND INTERNATIONAL APPROVALS

Section 2.1 Previous Australian Releases

16. A number of limited and controlled releases (field trials) have been previously approved in Australia. The aim of these trials was to assess the agronomic performance of all seven lines and their behaviour in the Australian environment. Fourteen limited and controlled releases of the lines were conducted under the voluntary system overseen by GMAC, as listed below:
- Male sterile and fertility restorer lines (RF1, RF2, RF3, MS1 and MS8), including the resultant hybrids such as InVigor[®] (MS8 x RF3), PR-63, PR-63X, PR-63X(2), PR-63X(3), PR-63X(4), PR-63X(5), PR-63X(6) and PR-63X(7), and
 - Glufosinate ammonium tolerant open-pollinated lines T45 and Topas 19/2, PR-62, PR-62X, PR-62X(2), PR-62X(3), PR-62X(4) and PR-62X(5).
17. The first release in Australia of lines covered by this application was in 1996. All previous releases have been carried out under conditions to limit spread or persistence of the GMO in the environment. The lines have been grown in various Australian locations and conditions in New South Wales, Victoria, South Australia, Western Australia, Queensland and Tasmania to select the best varieties for further development. In the largest approved trial, the planting area was 712 hectares. No adverse effects on human health and safety or the environment were reported for any of these releases.

18. On 30 July 2002 the Regulator issued a licence (DIR 010/2001) to Bayer for a limited and controlled release of InVigor[®] canola on 90 sites in 23 shires in New South Wales, Victoria, Western Australia and South Australia comprising a total area of 318 hectares over 3 years (106 hectares per annum).
19. The approvals issued by GMAC and the Regulator included conditions for the management of the trials to minimise the risks posed by the GM canola. Monitoring undertaken by the IOGTR identified a number of instances of non-compliance with GMAC conditions, as detailed in IOGTR Quarterly Reports (IOGTR 2000a; IOGTR 2000b; IOGTR 2001b).
20. Some of these instances of non-compliance related to trials of InVigor[®] canola, specifically PR-63X(4) (IOGTR 2000a; IOGTR 2001b) and PR-62X(4) (IOGTR 2001b). In some of these instances Bayer notified the IOGTR of the non-compliance. No instances of non-compliance were subsequently identified by the IOGTR or the OGTR for the reporting periods April-June 2001 (IOGTR 2001a) and July-September 2001 (OGTR 2002d). There were no instances of non-compliance in the periods October-December 2001 (OGTR 2002f), January-March 2002 (OGTR 2002c), April-June 2002 (OGTR 2002b), and July-September 2002 (OGTR 2002e).
21. Most of the instances of non-compliance related to post-harvest monitoring licence conditions, in particular the requirement to remove volunteer canola from the trial site prior to flowering. In each instance GMAC and the IOGTR/OGTR assessed the risks posed to human health and safety or the environment as a result of the non-compliances as negligible. Bayer also undertook management actions to further minimise any risks, including the removal of volunteers, destruction of the current crop on the site and extension of the monitoring period for non-compliant sites.
22. Studies commissioned by the OGTR on gene flow from non-compliant sites did not demonstrate any gene flow to other Brassicaceous species (Rieger 2002; Agronico 2002). There have been no observed adverse effects on human health and safety or the environment from these incidents.
23. Organisations are also required to provide monthly monitoring data to the OGTR. In October and November 2002, Bayer provided a number of monitoring reports to the OGTR in regard to former GM canola sites in Tasmania at which flowering volunteers had been identified. The sites of concern were: PR-63X(4), Site 73 PR-62X(4), Site 14 PR-62X(4), Site 13. These sites were in post harvest monitoring phase, ie the GM canola had been harvested and 'post-harvest' crops (not canola) had been sown.
24. A risk assessment conducted by the OGTR determined that at two of these sites detection of volunteer GMO canola was difficult due to the cover crops and could lead to a risk of persistence of the GMO in the environment and its possible dissemination. Bayer arranged to destroy the post harvest crops at the two sites to allow for control of volunteer plants. The OGTR determined that continued monitoring by Bayer at the remaining site would allow for adequate control of GM volunteer canola growth.

Section 2.2 Approvals by Other Australian Government Agencies

25. The OGTR is responsible for assessing the biosafety risks to human health and the environment associated with development and use of GMOs. Other government regulatory requirements must also be met in respect of the release of the GMOs, and the use of products of the GMO, including the requirements of the APVMA (formerly NRA) and FSANZ.

2.2.1 Food Standards Australia New Zealand (FSANZ)

26. The safety and labelling of foods derived from genetically modified plants are the responsibility of FSANZ, rather than the OGTR.
27. Only canola oil is consumed by humans in Australia (OGTR, 2002). FSANZ (formerly ANZFA) have approved the use of oil derived from the glufosinate ammonium tolerant male sterile, fertility restorer and resultant hybrid lines 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 (see Appendix 2).
28. Further details of the risk analysis conducted by FSANZ on lines T45, Topas 19/2, MS1, RF1, RF2, RF3 and MS8 and information about food labelling are available from FSANZ:

Food Standards Australia New Zealand
PO Box 7186 Canberra Mail Centre ACT 2610
Phone: (02) 6271 2222
Fax: (02) 6271 2278
E-mail: info@foodstandards.gov.au
<http://www.foodstandards.gov.au>

2.2.2 Australian Pesticides and Veterinary Medicines Authority (APVMA)

29. The registration of herbicides is the responsibility of the Australian Pesticides and Veterinary Medicines Authority (APVMA), formerly known as the National Registration Authority (NRA), rather than the OGTR.
30. Bayer has been granted registration of glufosinate ammonium for use on InVigor[®] canola under the trade name Liberty[®]. The APVMA has registered Liberty[®] for use only on InVigor[®] canola crops. Glufosinate ammonium is not registered for use in any other broad-acre cropping in Australia. Glufosinate ammonium is also registered as Basta[®] for weed control in horticultural and viticultural crops and Finale[®] for weed control in non-crop agricultural areas, commercial and industrial areas and rights-of-way. Appendix 4 contains further details.
31. Further information about the use and safety of insecticides and herbicides can be obtained from:

Australian Pesticides and Veterinary Medicines Authority (APVMA)
PO Box E240 KINGSTON ACT 2604
Phone: (02) 6272 5158
Fax: (02) 6272 4753
Email: contact@apvma.gov.au
<http://www.apvma.gov.au>

Section 2.3 International Approvals for the seven canola lines

32. The seven lines included in the application lines have been approved for food (Table 3), feed (Table 4) and environmental (Table 5) safety in a number of countries.

Table 3: Food regulatory approvals obtained for the seven lines.

Country	Event	Year Approved
Australia	MS1/RF1/RF2/MS8/RF3	2002
Australia	T45	2002
Australia	Topas 19/2	2002
Belgium	MS1/RF1	1995
Canada	MS1/RF1	1994
Canada	MS1/RF2	1995
Canada	MS8/RF3	1997
Canada	T45	1997
Canada	Topas 19/2	1995
Europe	MS8/RF3	Submitted
Japan	MS1/RF1	1996
Japan	MS1/RF1/RF2	1997
Japan	MS8/RF3	1997
Japan	T45	1997
UK	MS1/RF1	1995
UK	MS1/RF2	1995
UK	Topas 19/2	1995
USA	MS1/RF1	1996
USA	MS1/RF2	1996
USA	MS8/RF3	1998
USA	T45	1997
USA	Topas 19/2	1995

Table 4: Feed regulatory approvals obtained for obtained for the seven lines.

Country	Event	Year Approved
Belgium	MS1/RF1	1996
Canada	MS1/RF1	1995
Canada	MS1/RF2	1995
Canada	MS8/RF3	1996
Canada	T45	1996
Canada	Topas 19/2	1995
Japan	MS1/RF1	1996
Japan	MS1/RF2	1997
Japan	MS8/RF3	1998
Japan	T45	1997
Japan	Topas 19/2	1996
Mexico	Topas 19/2	1998
UK	MS1/RF1	1995
UK	Topas 19/2	1996
USA	MS1/RF1	1996
USA	MS1/RF2	1996

USA	MS8/RF3	1998
USA	T45	1997
USA	Topas 19/2	1995

Table 5: Environmental regulatory approvals obtained for the seven lines.

Country	Event	Year Approved
Canada	MS1/RF1	1995
Canada	MS1/RF2	1995
Canada	MS8/RF3	1996
Canada	T45	1996
Canada	Topas 19/2	1995
Europe*	MS1/RF1	1996 & 1997
Europe*	MS1/RF2	1997
Europe*	MS8/RF3	Submitted
Europe/UK	Topas 19/2	1998
Japan (import only)	MS1/RF1	1996
Japan (import only)	MS1/RF2	1997
Japan	MS8/RF3	1998
Japan	T45	1997
Japan	Topas 19/2	1998
UK*	MS1/RF1	1996
UK*	T45	Submitted
UK*	Topas 19/2	1996
USA	T45	1998
USA	MS8/RF3	1999
USA	MS1/RF1/RF2	2002
USA	Topas 19/2	2002

* European Union countries currently have a moratorium on commercial cultivation of GM crops

33. The Bayer canola lines MS8 and RF3, and their hybrid (MS8 x RF3) have been approved for growing and consumption in the US, Canada and Japan and their environmental and food safety are currently being assessed by regulators in Europe. The Scientific Committee on Plants of the European Commission concluded that InVigor[®] canola was unlikely to cause adverse effects on human health and the environment (European Scientific Committee on Plants 1998b).
34. The Belgian Government has refused to approve an application from Bayer to conduct field tests with GM herbicide tolerant canola (Minister for Consumer Interests Health & Environment 2002). In the communique on the decision, the Belgian Minister noted that pollen may be transferred up to 4 km by bees and that there was therefore a chance of dissemination of the GMO even with containment measures and that in their assessment there was uncertainty regarding possible effects on the environment. The issue of pollen transfer is considered in detail in Appendix 5. Subsequent advice from the Service of Biosafety and Biotechnology (Service of Biosafety and Biotechnology (SBB) 2003) indicated that the Minister's decision included considerations of adventitious presence of GM canola in the surrounding farms or in honey and isolation from nature reserves.
35. No other country is known to have refused an application for the release of glufosinate ammonium tolerant male sterile, fertility restorer and the hybrid lines on the basis of risks to human health and safety or the environment. The commercial use of GM

canola lines RF1, MS1 and Topas 19/2 was not allowed in France or Greece through those states invoking Article 16 of the European Union Directive 90/220/EEC. However the European Scientific Committee on Plants assessed each of these cases and did not consider that the information submitted supported a ban on the basis of risks to human health and safety or the environment (European Scientific Committee on Plants 1999c, 1999d, 1999e; The European Commission 2003).

36. There have been no reports of adverse effects on human health or the environment resulting from the use or release of any of the seven canola lines in Australia or any other countries in which they have been approved.

CHAPTER 2 SUMMARY OF THE RISK ASSESSMENT AND THE RISK MANAGEMENT PLAN

37. The *Gene Technology Act 2000* (the Act) and associated Regulations require that risks associated with dealings with GMOs are identified and assessed as to whether they can be managed to protect human health and safety or the environment (see Appendix 9).

SECTION 1 ISSUES RAISED IN CONSULTATION ON THE APPLICATION AND THE RARMP

38. Comments received from expert groups and key stakeholders consulted on application DIR 021/2002, as required by Section 50 of the Act, and on the risk assessment and risk management plan (RARMP) from the same organisations and the public, as required by section 52 of the Act (see Appendix 9), were very important in finalising this document which formed the basis of the decision on the application.
39. Written submissions on the application from the agencies and authorities prescribed by Section 50 of the Act, and other interested organisations that were consulted by the Regulator, suggested a number of issues relating to the protection of human health and safety and/or the environment that were taken into account, in accordance with Section 51 of the Act, in preparing the consultation version of the RARMP. These comprised:
- the molecular basis for function and specificity of the herbicide tolerance protein produced by InVigor[®] canola (Appendix 1 refers);
 - whether food products from this GM canola may be harmful to humans, as a result of toxicity or allergenicity (Appendix 2 refers);
 - the toxicity of introduced proteins to organisms other than humans (Appendix 3 refers);
 - the potential weediness of InVigor[®] canola (Appendix 4 refer);
 - the extent of cross-pollination and gene flow from InVigor[®] canola to other canola crops (Appendix 5 refers);
 - the potential for cross-pollination and gene flow with other Brassica and weedy brassicaceous species (Appendix 5 refers); and
 - whether the new genes introduced into the canola can transfer to other organisms with adverse consequences (Appendix 5 refers).
40. Submissions received from the consultation on the RARMP, as required by Section 52 of the Act (which included a two month period for public comment), also raised a range of issues. Those relating to food safety and herbicide usage, as explained in Chapter 1 Section 2.2, are the responsibility of FSANZ and the APVMA respectively. Others fell outside the scope of the assessment process and are discussed in Section 2 of this chapter.
41. All issues relating to risks to human health and safety and environment were covered in the consultation version of the RARMP. However, recognising the complexity of some of the issues, considerable sections of the finalised plan have been reviewed and expanded to further explain the evaluation process and the basis of the conclusions reached. The main areas where this has occurred are as follows:

ISSUE	ENHANCED EXPLANATION
Clarification of the conclusions of the weediness appendix	Environmental safety - Weediness: Appendix 4 Section 3
Further consideration of the seedbank of GM canola due to harvest loss and post harvest management conditions	Environmental safety - Weediness: Appendix 4 Section 2.1 and 2.2
Further clarification of the responsibilities of the APVMA and the Gene Technology Regulator with respect to herbicide resistance management	Regulation of herbicides: Chapter 1 Section 2.2.2; Appendix 6 Sections 1 and 2
Explanation of the APVMA's process for registering of glufosinate ammonium for use on InVigor® canola	Regulation of herbicides Chapter 1 Section 2.2.2; Appendix 6 Sections 1 and 2
Further consideration of hybrid vigour in terms of weediness of the GM canola and in terms of gene transfer	Environmental safety - Gene transfer Appendix 4 Sections 2.2 and 3; Appendix 5 Section 1.2.3
Reconsideration of the likelihood and impact of introgression into related Brassicaceous weeds	Environmental safety - Gene transfer Appendix 5 Sections 2.2.2, 3, 2.3, 2.3.2 and 2.3.3
Further consideration of the risks associated with gene transfer to <i>Brassica rapa</i> , including the incorporation of recent research findings	Environmental safety - Gene transfer Appendix 5 Sections 2.2.2, 2.3 and 2.3.2
Further consideration of the risks associated with gene transfer to <i>Raphanus raphanistrum</i> , including the incorporation of recent research findings	Environmental safety - Gene transfer Appendix 5 Sections 2.2.3, 2.3 and 2.3.2
Further clarification of issues that represent economic impacts on agriculture	Regulatory scope Appendices 4 and 5

42. In total, the OGTR received 256 written submissions from the public on this risk assessment and risk management plan. A detailed analysis is provided in Appendix 10.
43. Some raised queries about the suitability of the applicant to hold a licence for the release. Issues considered when assessing the suitability of the applicant are discussed in Section 4 of this chapter and Section 6 of Appendix 8.
44. Public submissions also raised a number of issues, such as impacts on domestic and export markets, marketing, costs and adequacy of segregation protocols, liability and impacts on organic status. As explained in Section 2, these are outside the scope of the evaluations conducted under the Act and have therefore not been considered as part of the assessment process.

SECTION 2 INDUSTRY MANAGEMENT PROPOSALS

45. A number of submissions expressed concern about the possible impact of the commercial release of GM canola on non-GM crops and markets *e.g.* the status of Australian grain exports. Some queried why proposed industry management strategies were not included in the licence conditions.

Section 2.1 Assessment of industry management proposals

46. The GTGC Canola Industry Stewardship Protocols for Coexistence of Production Systems and Supply Chains and the applicant's InVigor[®] Canola Crop Management Plan were both mentioned but not included in the original application from Bayer. In the absence of this material, the Regulator was not able to fully assess or make a judgement about the possible risks posed by the commercialisation of the genetically modified canola. Therefore the Regulator 'stopped the clock' on the application until this material was provided.
47. These documents were provided to the Regulator in late December 2002 and were subsequently assessed in detail. Summaries of the key elements of these documents are outlined in Appendix 7.
48. The proposed industry management strategies promote good agricultural practice in relation to seed purity, cultivation, handling, transport etc. They are designed to preserve the use of Bayer's technology and enable segregation of GM and non-GM canola to the level required by markets, rather than total separation.
49. The potential for some mixing and dissemination of GM canola to occur in the supply chain is acknowledged. However, the assessment by the Regulator concluded that this would not pose any additional risks to human health and safety or the environment to the dealings proposed in the application - which do not anticipate any containment measures, such as buffer zones (i.e. the risk assessment process considered the risks that might occur in the absence of supply chain management controls).

Section 2.2 Role of State and Territory Governments

50. It is important to note that the evaluation of trade, marketing and cost/benefit issues have been intentionally excluded from the Gene Technology Act 2000 assessment process. Feedback from the extensive public consultation process that led to the development of the legislation identified concerns that a requirement for the Regulator to consider such issues had the potential to compromise the regulatory system's focus upon the scientific evaluation of risks, and the protection of human health and safety and the environment. Therefore, this RARMP cannot draw any conclusions about the possible costs or benefits of the Bayer canola to individual farmers, or on market impacts for the agricultural industry.
51. However, these issues are being actively considered by the Commonwealth, State and Territory Governments. The Primary Industries Ministerial Council, which has members from all Australian jurisdictions, has indicated its view that the introduction of GM crops is a matter for industry self-regulation, with oversight by government.
52. The Act itself anticipates that State and Territory Governments may take action to declare "GM or non-GM designated areas for marketing purposes". The Gene Technology Ministerial Council, which has similar representation, is in the process of issuing a policy principle to recognise such areas.
53. A number of State governments have initiated voluntary or legislative measures to ensure the orderly and phased introduction of GM canola into the Australian market. These measures involve further examination of the proposed industry segregation procedures and consideration of market effects.

54. Although these arrangements in no way preclude the Regulator approving the commercial release of InVigor[®] canola on health and environmental grounds they may influence the rate of take-up of this product.

SECTION 3 FINALISATION OF RISK ASSESSMENT & RISK MANAGEMENT PLAN

Section 3.1 Risk Assessment process

55. In accordance with Section 51 of the Act, the Regulator has taken into account all issues raised in written submissions that related to human health and safety and to the environment in finalising the risk assessment and the risk management plan. These issues were considered carefully and weighed against the body of current scientific information in reaching the conclusions set out in this document.
56. The risk assessment process, detailed in Appendix 9, identified a number of potential hazards that may be posed by the proposed dealings. The risks posed by these hazards were assessed as being either 'negligible', 'very low', 'low', 'moderate', 'high' or 'very high', by considering:
- the likelihood of the hazard occurring
 - the likely consequences (impact) of the hazard, were it to be realised and
 - risk management options to mitigate any significant hazards.
57. Table 1 at the end of this Chapter lists each of the potential hazards that were considered during the risk assessment process in the Hazard Identification column and summarises the assessment of each hazard under the column headed Risk Assessment. A comprehensive assessment of each identified hazard is provided in Appendices 2 - 6, as cross-referenced in the column headed Summary Justification of Risk Assessment.

Section 3.2 Risk Management Considerations

58. In assessing the application for the commercial release of InVigor[®] canola, the Regulator considers the need to impose conditions to manage any risks to human health and safety or the environment. This includes consideration of whether any conditions would be effective in managing risks, particularly if it was considered necessary to contain the GMO given the widespread scale of the proposed commercial release and the outcrossing nature of canola. The assessment also includes consideration of whether any conditions imposed could be effectively implemented and compliance monitored and enforced.
59. Given the widespread scale and ongoing nature of a commercial release, the Regulator considers that the release should only be approved if the risks to human health and safety or the environment are low to non-existent and therefore do not require a range of specific licence conditions for them to be managed.
60. In considering the commercial release of InVigor[®] canola, there are a number of aspects of the release which the Regulator must be satisfied pose low risks to human health and safety or the environment. These include:
- the ability of canola to outcross at low levels over considerable distances;
 - the ability of canola seed to remain dormant in the seedbank and germinate as volunteers; and

- the fact that segregation procedures proposed by industry may limit mixing of GM and non-GM seed to low levels but may not eliminate it entirely.
61. The risk management plan summarised in Table 1 concludes that, irrespective of whether segregation supply chain management measures are introduced by industry for marketing purposes, the risks are considered low to negligible and therefore do not require specific licence conditions for them to be managed.
 - 62.
 63. In finalising the conditions for the licence, the Regulator has carefully considered the enforceability of the conditions and the ability for the licence holder and persons covered by the licence to comply with the conditions in practice.
 64. The Regulator will continue to proactively review any new information about risks of the release and may amend or add licence conditions accordingly. Under section 68 of the Act, the Regulator may also suspend or cancel a licence if a licence has been breached or if the Regulator becomes aware of new risks that are not adequately managed. The Regulator can also vary a licence to impose extra management conditions if necessary.

SECTION 4 DECISION ON THE APPLICATION

65. Details of the matters that the Regulator must consider in making a decision are provided in Appendix 9. It is important to note that the legislation requires the Regulator to base the licence decision on whether risks posed by the dealings can be managed so as to protect human health and safety and the environment.
66. It is concluded that there are no risks to public health and safety or to the Australian environment arising from the proposed release of GM canola lines T45, Topas 19/2, RF1, RF2, RF3, MS1 or MS8 that are additional to the low risks posed by the commercial production of conventional canola (noting that Bayer has indicated that it only intends to commercialise lines RF3, MS8 and their hybrids as InVigor[®] canola). Detailed risk analyses based on the available scientific information are provided in Appendices 2 - 6 in support of this conclusion.
67. A range of segregation measures have been proposed by the applicant company and industry bodies to facilitate co-existence between GM and non-GM canola production systems for marketing purposes. However, in the absence of evidence of risks to human health and safety or the environment from the proposed release, no specific supply chain management conditions have been included in the licence conditions for the commercial release of GM canola lines T45, Topas 19/2, RF1, RF2, RF3, MS1 and MS8 canola.
68. The Regulator must also be satisfied that Bayer CropScience Pty Ltd is a suitable applicant to hold a licence, and must have regard to the matters prescribed by section 58 of the Act. These include any relevant convictions, any revocations or suspensions of licences or permits in Australia or overseas, and the capacity of Bayer CropScience Pty Ltd to meet the conditions of the licence (further information on the process of assessing the suitability of the applicant is contained in Appendix 9).
69. All strategic decisions affecting Bayer CropScience Pty Ltd reside with Bayer CropScience Pty Ltd Australian management. Based on the compliance history of Bayer CropScience Pty Ltd in Australia, and the effective management and control of

the Australian operations, the Regulator considers Bayer CropScience Pty Ltd is suitable to hold the licence.

70. Having taken all of the above requirements into account, the Regulator has issued licence number DIR 021/2002.

Table 1 Summary of the risk assessment and the risk management plan for DIR 021/2002

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
TOXICITY OR ALLERGENICITY FOR HUMANS		<p>See Appendix 2</p> <p>Canola oil is the only fraction used as human food. Canola seed or meal is not used in human food; Food Standards Australia New Zealand (FSANZ) has approved the use of oil derived from all seven GM canola lines in human food. The potential for human exposure is negligible, as canola oil does not contain protein or DNA.</p>
<i>Toxicity</i>	Negligible	<p>Toxicology studies indicate that the GM canola lines are no more toxic than conventional canola; Acute oral toxicity studies demonstrate that the PAT and NPTII proteins are not toxic, even at high doses; Feeding studies in a range of animals demonstrate that there are no toxic or anti-nutritional effects of the genetic modifications in the GM canola; The proteins produced by the introduced genes, PAT, Barnase, Barstar and NPTII, are not similar to any known toxins; Compositional analyses of the seven GM canola lines show no significant differences to non-GM canola as a result of the genetic modifications; The levels of the naturally occurring toxicants of canola, erucic acid and glucosinolates, do not vary between GM and non-GM canola; and The major metabolites of the glufosinate ammonium herbicide are not toxic.</p>
Allergenicity	Negligible	<p>Allergenicity is unlikely because humans are frequently exposed to all of the novel proteins because they are derived from common bacteria that are naturally ubiquitous in the environment; All of the novel proteins, PAT, Barnase, Barstar and NPTII are expressed at low or very low levels, do not share significant sequence homology with known protein allergens, and are all rapidly degraded by mammalian digestive systems; The Barnase and Barstar proteins are only expressed in specific cells in developing flowers. Expression of the introduced genes is not detected in pollen; Only the PAT protein is expressed in canola seed; and The NPTII protein is not expressed in the RF3 or MS8 lines intended for commercialisation in Australia.</p>
TOXICITY OR ALLERGENICITY FOR OTHER ORGANISMS		<p>See Appendix 3</p> <p>The fact that proteins produced by the introduced genes, PAT, Barnase, Barstar and NPTII, are naturally occurring in soil and water organisms, are expressed at low levels, and are not toxins or allergens, together with evidence that the composition of the plants has not changed significantly, strongly support the conclusion that the GM canola will not present any toxicity or allergenicity hazard to vertebrates, invertebrates, microbes and soil biota.</p>

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
Vertebrates, including grazing animals, birds and native animals	Negligible	<p>All of the novel proteins, PAT, Barnase, Barstar and NPTII are expressed at low or very low levels in any plant tissues, do not share significant sequence homology with known protein toxins or allergens, and are all rapidly degraded by mammalian digestive systems;</p> <p>The levels of the naturally occurring toxicants of canola, erucic acid and glucosinolates, do not vary between GM and non-GM canola;</p> <p>Nutritional composition of the plants does not vary between GM and non-GM canola;</p> <p>The major metabolites of the glufosinate ammonium herbicide are not toxic;</p> <p>Only the PAT protein is expressed in canola seed;</p> <p>Feeding studies in a range of animals, including rabbits, broiler chickens, canaries demonstrate that there are no toxic or anti-nutritional effects of the genetic modifications in the GM canola;</p> <p>The digestibility and nutritional value of the GM canola seed is not different to conventional canola;</p> <p>Feeding studies in pigs and sheep with other glufosinate ammonium tolerant GM crop plants also demonstrate that there are no anti-nutritional effects associated with the presence of the PAT protein;</p> <p>PAT protein present in GM canola seed will be destroyed by the normal processing of canola seed to produce meal for use in animal feed; and</p> <p>There are no reports of adverse effects of the GM canola lines on native animals or birds during trials in Australia or commercial production in North America.</p> <p>All these data support the conclusion that the GM canola will not be toxic to agricultural or native animals.</p>
Invertebrates, including insects	Negligible	<p>Floral and nectary development is normal in all seven GM lines and MSxRF hybrids;</p> <p>Pollen production is normal in GM canola lines T45, Topas 19/2, RF1, RF2 and RF3 and MSxRF hybrids; GM canola lines MS1 and MS8 lack anthers and do not produce pollen. MS lines are only grown alone for breeding and seed increase;</p> <p>There are no differences between the behaviour and health of bees foraging on the GM canola lines or non-GM canola;</p> <p>Studies with other GM glufosinate ammonium tolerant canola lines expressing the PAT protein have found no adverse impacts on foraging bees;</p> <p>No significant differences were found in the numbers of soil arthropods, including spiders and beetles, between RF1xMS1 hybrid GM canola and non-GM canola., nor did glufosinate ammonium application result in significant differences;</p> <p>There are no reports of adverse effects of the GM canola lines on invertebrates during trials in Australia or commercial production in North America.</p>
Soil biota	Negligible	<p>All of the introduced genes are derived from commonly occurring soil or commensal bacteria and the encoded proteins can be expected to already be present in soil;</p> <p>The Barnase protein is naturally excreted by <i>Bacillus amyloliquefaciens</i> but its expression in the GM canola plants is restricted to specific cells in developing flowers;</p> <p>The proteins produced by the introduced genes are expressed at low to very low levels in the GM canola plants;</p> <p>No significant differences have been detected in soil microbe populations between the GM canola lines and non-GM canola or any significant differences as a result of glufosinate ammonium herbicide application. Studies with other glufosinate ammonium tolerant GM canola lines (not included in this application) detected small differences in soil microbe populations relative to non-GM canola, but the observed differences were much less than population shifts that occur normally during plant development or differences in soil type. One study also noted small differences in the soil microbe population following herbicide application, either glufosinate ammonium or the metazachlor herbicide (mode of action unrelated to glufosinate ammonium or PAT protein);</p> <p>Studies with other glufosinate ammonium -tolerant GM crop plants also found no differences in soil microbes associated with the presence of the PAT protein, nor was there any observed effect of the application of glufosinate ammonium herbicide.</p>

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
<p>WEEDINESS Persistence in the Environment</p>	<p>See Appendix 4</p>	<p>Although conventional canola has a number of weedy characteristics, it is a poor competitor and is not invasive. Conventional canola is not a significant weed in habitats outside agricultural areas and does not pose a serious threat to the environment and biodiversity. The risk that the GM canola lines will be more likely to persist in the environment and cause more harm to the environment than conventional (non-GM) canola, is negligible.</p> <p>There is no evidence to show that the introduced genes increase the potential weediness of the plants. The genetic modifications do not provide the GM canola lines with an ecological advantage over conventional canola except in the presence of glufosinate ammonium. The germination, seed dormancy and fitness traits such as herbicide sensitivity, disease resistance, stress adaptation and competitiveness for the seven GM canola lines fall within the range of conventionally bred canola varieties.</p> <p>InVigor[®] canola hybrids derived from crossing RF and MS lines display superior seedling emergence and vigour, and increased seed yield and size. However, these and other life history characteristics are within the range exhibited by conventional hybrids and open pollinated canola. The hybrid vigour is not a direct result of the genetic modifications, but the male sterile (MS) and fertility restorer (RF) lines provide a means of ensuring hybrid seed.</p> <p>The APVMA has registered glufosinate ammonium as Liberty[®] for use in InVigor[®] canola crops. Glufosinate ammonium herbicide is currently not registered by APVMA for any other use in broad acre agriculture.</p> <p>Glufosinate ammonium is also used in horticulture and viticulture (registered as Basta[®]) and non-crop agricultural areas, commercial and industrial areas and rights-of-way (registered as Finale[®]) but is not a widely used chemical for this purpose.</p> <p>The GM canola lines are only tolerant to glufosinate ammonium and their susceptibility to other herbicides is no different to conventional canola. GM volunteers can be managed and controlled in the same manner as conventional canola volunteers.</p>
<p>Agricultural environments</p>	<p>Negligible</p>	<p>See Appendix 4</p> <p>The risk that the GM canola lines will be more likely than conventional (non-GM) canola to persist in the agricultural environment, and result in more detrimental environmental impact, is negligible.</p> <p>Conventional canola can persist as an agricultural weed, particularly as volunteers following canola crops;</p> <p>There are no differences between the GM canola lines and non-GM canola with respect to the intrinsic characteristics contributing to ecological persistence, such as seed production shattering or dormancy, and competitiveness.</p> <p>InVigor[®] hybrids derived by crossing RF and MS lines display hybrid vigour in the F1 generation. The vigour is within the range of that of hybrids derived through conventional breeding. It is these hybrids that will be cultivated, but the vigour will decline in subsequent generations and volunteers from InVigor[®] canola crops will not be any more weedy than conventional canola.</p> <p>The GM canola lines only have a survival advantage in the presence of glufosinate ammonium and are as susceptible to the herbicides currently used to control canola volunteers as conventional canola; and</p> <p>Glufosinate ammonium registered by APVMA as Liberty[®] may only be used on InVigor[®] canola crops not for weed control in other crop rotations.</p>

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
Non-cropped disturbed environments	Negligible	<p>See Appendix 4</p> <p>The risk that the GM canola lines will be more likely than conventional (non-GM) canola to persist in non-cropped disturbed environments, and result in more detrimental environmental impact, is negligible.</p> <p>Conventional canola is a minor weed of non-cropped disturbed environments such as roadsides, normally resulting from seed spillage during harvest and transport operations;</p> <p>Conventional canola does not tend to persist in these environments, and survey observations indicate it does not establish beyond the first few metres adjacent to roads and it is not a good competitor;</p> <p>GM canola volunteers occurring in disturbed environments will not have any competitive advantage over conventional canola in the absence of glufosinate ammonium selection;</p> <p>Glufosinate ammonium is registered for use in non-crop agricultural areas (Basta®), commercial and industrial areas and rights-of-way (Finale®) but is not a widely used in these areas; and GM canola volunteers can be controlled using other herbicides or non-chemical techniques currently used for weed control in disturbed environments.</p>
Undisturbed environments	Negligible	<p>See Appendix 4</p> <p>The risk that the GM canola lines will be more likely than conventional (non-GM) canola to persist in undisturbed environments, and result in more detrimental environmental impact, is negligible.</p> <p>Conventional canola is not considered a weed of undisturbed environments. It is not considered invasive and it does not persist in undisturbed environments;</p> <p>The GM canola will be no more likely to establish and persist in undisturbed environments than conventional canola. The GM canola lines do not have any competitive advantage in the absence of glufosinate ammonium; and</p> <p>If GM canola did occur in these environments it can be effectively controlled using other herbicides and non-chemical management techniques.</p>
WEEDINESS – spread in the environment	Negligible	<p>See Appendix 4</p> <p>The risk that the GM canola lines will be more likely than conventional (non-GM) canola to spread in the environment, and result in more detrimental environmental impact, is negligible</p> <p>Conventional canola is primarily dispersed by human activities (harvest, transport) and this would be the case with the GM canola lines;</p> <p>Conventional canola is non-invasive and considered a poor competitor.</p> <p>The GM canola lines do not differ from conventional canola in growth characteristics in terms of flowering period, pollen production and pollen viability (except in the male sterile lines), seed production, seed size, seed germination and dormancy and agronomic performance, including disease resistance potential and sensitivity to herbicides other than glufosinate ammonium; and</p> <p>Seed shattering ability, seed size and seed weight of each of the GM canola lines were no different to conventional canola lines indicating no alteration in the potential for seed dispersal.</p> <p>InVigor® canola hybrids derived from crossing RF and MS lines display superior seedling emergence and vigour, and increased seed yield and size, however the hybrid vigour manifested falls is within the range of vigour exhibited by conventional hybrids. Increased vigour will be manifested in the cultivated F1 generation.</p> <p>The genetic modifications do not make the seven GM canola lines or RFxMS hybrids more invasive or persistent in the environment.</p>

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
<p>GENE TRANSFER – Plants: other canola crops</p>	Negligible	<p>See Appendix 5</p> <p>Canola is mainly self-pollinated but outcrossing (approximately 30%) does occur; The highest rates of outcrossing are between adjacent plants (less than 5m), and the rate decreases significantly at distances of over 5-10m;</p> <p>Outcrossing can be detected at greater distances, but at extremely low levels –detected up to 2.6km under Australian conditions;</p> <p>In a commercial situation low levels of outcrossing between canola varieties is inevitable. If gene transfer from the GM canola lines to non-GM canola did occur, the hazards are the same as those for the GM canola lines;</p> <p>GM canola volunteers resulting from outcrossing events will not have any selective advantage in the absence of glufosinate ammonium;</p> <p>The APVMA has registered glufosinate ammonium as Liberty® for use in InVigor® canola crops. Glufosinate ammonium herbicide is currently not registered by APVMA for any other use in broad acre agriculture.</p> <p>The GM canola lines are only tolerant to glufosinate ammonium and their susceptibility to other herbicides is no different to conventional canola. GM volunteers can be managed and controlled in the same manner as conventional canola volunteers.</p>
<p><i>B. napus</i> vegetables and forage rape</p>	Negligible	<p>See Appendix 5</p> <p>Gene flow is possible from <i>B. napus</i> canola to <i>B. napus</i> forage rape and vegetables such as swedes, rutabaga and Siberian kale. However, gene transfer would require flowering synchrony and <i>B. napus</i> vegetables are generally harvested before flowering;</p> <p><i>B. napus</i> vegetable seed production crops are isolated from other <i>B. napus</i> vegetable or canola crops to prevent outcrossing;</p> <p>Forage rape crops rarely flower and are consumed prior to flowering or seed production;</p> <p>If outcrossing and introgression of the introduced genes from the GM canola lines did occur, the hybrid plants would not have any survival advantage in the absence of glufosinate ammonium herbicide;</p> <p>The APVMA has registered glufosinate ammonium as Liberty® for use in InVigor® canola crops. Glufosinate ammonium herbicide is currently not registered by APVMA for any other use in broad acre agriculture. Glufosinate ammonium is also used in horticulture and viticulture (registered as Basta®) and non-crop agricultural areas, commercial and industrial areas and rights-of-way (registered as Finale®) but is not a widely used chemical for this purpose. Glufosinate ammonium tolerant hybrids can be effectively controlled using alternative herbicides and other non-chemical management techniques currently used for the control of <i>B. napus</i> vegetables and forage rape.</p>
<p>GENE TRANSFER – Plants: related Brassica species</p>		<p>See Appendix 5</p> <p>Conventional canola can outcross and form inter-specific hybrids with closely related <i>Brassica</i> species <i>B. rapa</i>, <i>B. juncea</i> and to a lesser extent <i>B. oleracea</i>;</p> <p>Introgression (<i>ie.</i> incorporation of genes into a population after an outcrossing event) from canola to <i>B. rapa</i> and <i>B. juncea</i> can occur.</p>

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
<i>B. rapa</i>	Very low	<p><i>Brassica rapa</i> is a weed of disturbed and cultivated land and is not found in undisturbed habitats; <i>B. rapa</i> is a major weed in Tasmania but its incidence is concentrated in particular geographic locations</p> <p><i>B. rapa</i> is a minor weed of WA, SA, Qld, NSW and Vic;</p> <p>Inter-specific hybrids of canola and <i>B. rapa</i> have reduced fertility, seed set and fitness relative to their parents. However recent evidence suggests that hybrids may have increased female fitness and these factors will also be influenced by the frequency of parents and hybrids.</p> <p>Low levels of outcrossing and introgression of the introduced genes from GM canola to <i>B. rapa</i> populations is likely over time if they are in physical proximity and flower in synchrony;</p> <p>Due to the greater incidence of <i>B. rapa</i> in Tasmania than on the mainland, gene transfer and introgression may be more likely to occur in Tasmania.</p> <p>If outcrossing and introgression of the introduced genes from the GM canola lines did occur, the inter-specific hybrid plants would not have any survival advantage in the absence of glufosinate ammonium herbicide;</p> <p>The APVMA has registered glufosinate ammonium as Liberty® for use in InVigor® canola crops. Glufosinate ammonium herbicide is currently not registered by APVMA for any other use in broad acre agriculture. Glufosinate ammonium is also used in horticulture and viticulture (registered as Basta®) and non-crop agricultural areas, commercial and industrial areas and rights-of-way (registered as Finale®) but is not a widely used chemical for this purpose.</p> <p>Glufosinate ammonium tolerant hybrids can be effectively controlled using alternative herbicides and other non-chemical management techniques currently used for the control of Brassica weeds.</p>
<i>B. juncea</i>	Negligible	<p><i>Brassica juncea</i> is a weed of cultivated and disturbed environments and is not present in undisturbed environments;</p> <p><i>B. juncea</i> is an occasional agricultural weed in areas of NSW and Vic;</p> <p>Inter-specific hybrids of canola and <i>B. juncea</i> have reduced fertility and seed set;</p> <p>Low levels of outcrossing and introgression of the introduced genes from GM canola to <i>B. juncea</i> populations is likely over time if they are in physical proximity and flower in synchrony;</p> <p>If outcrossing and introgression of the introduced genes from the GM canola lines did occur, the inter-specific hybrid plants would not have any survival advantage in the absence of glufosinate ammonium herbicide;</p> <p>The APVMA has registered glufosinate ammonium as Liberty® for use in InVigor® canola crops. Glufosinate ammonium herbicide is currently not registered by APVMA for any other use in broad acre agriculture. Glufosinate ammonium tolerant hybrids can be effectively controlled using alternative herbicides and other non-chemical management techniques currently used for the control of Brassica weeds.</p>
<i>B. oleracea</i>	Negligible	<p><i>Brassica oleracea</i> is not a weed in Australia;</p> <p>Outcrossing from canola (conventional or GM) to <i>B. oleracea</i> is unlikely to occur as hybrids not readily formed; and</p> <p>Commercial <i>B. oleracea</i> crops (eg. cabbage) are harvested prior to flowering.</p>

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
<p>GENE TRANSFER Plants: other Brassicaceous weeds</p>	<p>See Appendix 5</p>	
<p>Raphanus raphanistrum</p>	<p>Very low</p>	<p><i>R. raphanistrum</i> occurs in WA, Vic, SA, Qld, NSW and Tas and is a major weed in cropping areas of southern Australia; Inter-specific crossing between canola (either conventional or GM) and <i>R. raphanistrum</i> occurs at extremely low levels; The frequency of hybridisation is lower when canola is the pollen donor, hybrids are most likely to occur in canola crops with the majority of seed removed at harvest. Inter-specific hybrids of conventional canola with <i>R. raphanistrum</i> have low vigour and fertility; Even if outcrossing occurs, evidence suggests that there are significant barriers to introgression of genes from canola to <i>R. raphanistrum</i> If outcrossing and introgression of the introduced genes from the GM canola lines did occur, the inter-specific hybrid plants would not have any survival advantage in the absence of glufosinate ammonium herbicide; The APVMA has registered glufosinate ammonium as Liberty® for use in InVigor® canola crops. Glufosinate ammonium herbicide is currently not registered by APVMA for any other use in broad acre agriculture. Glufosinate ammonium tolerant hybrids can be effectively controlled using alternative herbicides and other non-chemical management techniques currently used for the control of <i>R. raphanistrum</i>. <i>R. raphanistrum</i> has a natural tolerance to glufosinate ammonium in the Australian environment and therefore the transfer of the glufosinate ammonium-tolerance gene would not alter the options for control of this weed.</p>
<p>Hirschfeldia incana</p>	<p>Very low</p>	<p><i>H. incana</i> occurs in Qld, NSW, Vic, SA, Tas and WA and is present in disturbed areas of agricultural and native environments; <i>H. incana</i> is a minor weed in agricultural areas of Qld and NSW; Inter-specific crossing with canola (conventional or GM) is very unlikely to occur; Inter-specific hybrids of conventional canola with <i>H. incana</i> have low vigour and fertility; <i>H. incana</i> possesses genes that inhibit homeologous pairing of chromosomes resulting in the expulsion of <i>B. napus</i> chromosomes in inter-specific hybrids;</p> <p><i>If outcrossing and introgression of the introduced genes from the GM canola lines did occur, the inter-specific hybrid plants would not have any survival advantage in the absence of glufosinate ammonium herbicide;</i></p> <p>The APVMA has registered glufosinate ammonium as Liberty® for use in InVigor® canola crops. Glufosinate ammonium herbicide is currently not registered by APVMA for any other use in broad acre agriculture; Glufosinate ammonium tolerant inter-specific hybrids can be effectively controlled using alternative herbicides and other non-chemical management techniques currently used for the control of this weed</p>

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
<i>Sinapis arvensis</i>	Very low	<p><i>S. arvensis</i> occurs in Qld, Vic, SA, NSW, Tas and WA; <i>S. arvensis</i> is a weed of cropped and non-cropped disturbed agricultural areas, particularly in cropping regions of NSW; Inter-specific crossing with canola (conventional or GM) is very unlikely to occur; Inter-specific hybrids of conventional canola with <i>S. arvensis</i> have low vigour and fertility; If outcrossing and introgression of the introduced genes from the GM canola lines did occur, the inter-specific hybrid plants would not have any survival advantage in the absence of glufosinate ammonium herbicide; Glufosinate ammonium herbicide is currently not registered by APVMA for any other use in broad acre agriculture; Glufosinate ammonium tolerant inter-specific hybrids can be effectively controlled using alternative herbicides and other non-chemical management techniques currently used for the control of this weed.</p>
GENE TRANSFER - Other organisms		See Appendix 5
<i>Humans</i>	Negligible	<p>Canola oil is the only fraction used as human food; The potential for human exposure to the introduced genes in the GM canola is low as canola oil does not contain DNA or protein; Food Standards Australia New Zealand (FSANZ) has approved the use of oil derived from all seven GM canola lines in human food; There is no evidence of the transfer and incorporation of DNA from plants to animals despite humans/animals ingesting large amounts of foreign DNA throughout evolutionary history; The likelihood of transfer of the introduced genes from the GM canola to humans is negligible; and Even if gene transfer did occur there would be no adverse consequences, all of the genes are derived from common bacteria and do not encode toxins or allergens.</p>
<i>Other Animals</i>	Negligible	<p>There is no evidence of the transfer and incorporation of DNA from plants to animals despite humans/animals ingesting large amounts of foreign DNA throughout evolutionary history; The likelihood of transfer of the introduced genes from the GM canola to animals is negligible; and Even if gene transfer did occur there would be no adverse consequences, all of the genes are derived from common bacteria and do not encode toxins or allergens.</p>
<i>Microorganisms (bacteria, viruses and fungi)</i>	Negligible	<p>Transfer of the introduced genes from the GM canola to microorganisms is extremely low; Transfer of DNA from GM plants to soil bacteria has been demonstrated but only under highly artificial laboratory conditions, between homologous sequences and under conditions of selective pressure and at very low frequency; Transfer of DNA from GM plants to soil bacteria has not been demonstrated under natural conditions; Transfer of DNA from GM plants to gut bacteria has not been demonstrated under experimental or natural conditions; Transfer of DNA from GM plants to plant viruses has only been demonstrated under controlled conditions between homologous sequences and under conditions of selective pressure and at very low frequency; and Transfer of DNA from GM plants to fungi has not been demonstrated under experimental or natural conditions. Even if gene transfer did occur there would be no adverse consequences, all of the genes are derived from common bacteria and do not encode toxins or allergens.</p>

APPENDIX 1 INFORMATION ABOUT THE GMO

71. In preparing the risk assessment and risk management plan, the Gene Technology Regulator is required under Section 49 (2) of the Gene Technology Act 2000 to consider the properties of the parent organism and the effects of genetic modification.
72. This part of the document addresses these matters and provides detailed information about the GMOs proposed for release, the parent organism, the genetic modification process, the genes that have been introduced and the new proteins that are expressed in the genetically modified canola.
73. Further information and analysis of the properties of the parent organism are contained in the reference document that was prepared by the OGTR entitled *The Biology and Ecology of Canola (Brassica napus)* (OGTR 2002a). This document is available from the OGTR website at www.ogtr.gov.au.
74. It should be noted that some detailed technical information on precise gene constructs and molecular characterisation data submitted to the Regulator in the original application and during the assessment process has been declared as Confidential Commercial Information (CCI) under Section 185 of the Act. However the CCI was made available to the prescribed expert groups which were consulted in the preparation of the risk assessment and risk management plan and this declaration in no way limited the thorough risk assessment of the individual genetically modified organisms.

SECTION 1 SUMMARY INFORMATION ABOUT THE GMO

Breeding hybrid plants

75. Traditional breeding selects for plants with agronomically valuable characteristics but over time can also result in highly inbred plants that do not grow or yield as well as non-inbred plants.
76. Hybrid crops are commercially very important in agriculture. A hybrid, which is the result of a cross between two different varieties of the same crop plant (for example, two different varieties of corn) can counteract this problem. Hybrid plants are often taller, produce more leaves and seeds (and thus higher yields), and are generally more vigorous than conventional varieties. In order to produce the large quantity of seed needed for sales to farmers, plant breeders must be able to make controlled crosses between the two varieties on a very large scale.
77. Plant reproduction is similar to animal reproduction. A flower, which contains the plant's sexual organs, produces pollen (the "sperm") which fertilises an ovule (the "egg") which will in turn develop into the next-generation plant (the seed contains the young plant embryo of the next generation). One key difference in plants is that many plants contain both the male and female parts on the same plant, even in the same flower, and such a plant can fertilise itself. Because of this important difference, the trick in producing a hybrid is to get one plant to fertilise a second plant without the second plant fertilising itself. In order to do this the plant breeder must in some way prevent the second plant from producing its own pollen.
78. Several pollen control techniques are used by breeders in hybrid production. One involves removal of the male parts of the flower by hand before they shed pollen - a process called emasculation. On corn plants, the male (tassel) and female (ear) parts are separate, so the plant can be emasculated simply by cutting off the tassel. Emasculation

in many other plants is much more tedious - often requiring tweezers and a magnifying glass! The methods are very labour-intensive, and make it very difficult to produce hybrid seed on a large scale.

79. There are also some natural genetic mechanisms that breeders can use to create 'male sterile' plants, but they are not available for many crops, and require additional steps to restore fertility in the hybrid (Genetically Engineered Organisms - Public Issues (geopie) 2003).

Genetic modification to facilitate hybrid breeding

80. Scientists have developed a novel way of making male sterile (MS) plants through genetic engineering, using a two-gene system from the soil bacterium *Bacillus amyloliquefaciens*. In nature, the bacterium excretes a defence protein called Barnase (RNase) which degrades the RNA of potential enemies (Hartley 1989). The bacterium protects itself from Barnase by producing a second protein called Barstar (RNase inhibitor), which binds with Barnase and renders it inactive. MS plants are engineered by making them produce Barnase in the pollen-producing tissues, blocking pollen production. A second 'restorer of fertility' (RF) line is engineered to express Barstar. When pollen from the Barstar plant is used to fertilise the male-sterile Barnase plant, the resulting hybrid progeny will be fully fertile, because they will express both the Barstar and Barnase proteins (Mariani et al. 1992).
81. So, hybrid vigour is not a direct result of the genetic modification, ie it is not encoded by a gene construct that can be transferred to other plants in the way that herbicide tolerance can. Rather the MS/RF breeding system ensures that the MS lines are obligate outcrossers that can only produce hybrid seed.

The GMOs

82. The GM canola seed which Bayer seeks to commercialise in Australia as InVigor[®] canola is based on this novel hybrid generation system. Regulatory approval was sought for seven (7) genetically modified lines of canola: T45 (synonym HCN28), Topas 19/2 (synonyms HCN92, Innovator), MS1 (synonym 91-4), RF1 (synonym 93-101), RF2 (synonym 94-2), RF3 and MS8. (The term 'line' has been used throughout this risk assessment and has been used to denote canola with a specific genetic modification derived from a single transformation event). Lines MS1, MS8, RF1, RF2 and RF3 and hybrids derived from MS x RF crosses are covered by the registered trade name InVigor[®] canola.
83. Bayer have indicated that it only intends the commercial release of InVigor[®] canola lines RF3, MS8, and MS8xRF3 hybrids in Australia. Only seed of MS8x RF3 hybrids would be grown by farmers in general commercial cropping, while the MS8 and RF3 lines would only be used in seed production and breeding activities.
84. However, Bayer is seeking approval for all seven lines to achieve consistency with existing Australian and overseas regulatory approvals.
85. It should be noted that the descriptor 'line' has been used throughout the risk assessment to denote GM canola with a specific genetic modification derived from a single transformation event (eg line MS1), but that this usage is intended to be inclusive of the introduction of the modification into other canola genetic backgrounds by conventional breeding.

86. The genetic modifications introduced into the various GM canola lines are summarised in Table 1. Five of the GM canola lines, RF1, RF2, RF3, MS1 and MS8, have been modified to introduce a novel hybrid breeding system for canola, based on genetically modified male sterile (MS) and fertility restorer (RF) lines. All seven of the GM canola lines have been genetically modified to introduce tolerance to the herbicide glufosinate ammonium. Four of the seven lines have also been modified by the introduction of an antibiotic resistance marker gene.

Table 1: Introduced genes in the seven GM canola lines

Line	Parental canola cultivar	Glufosinate ammonium tolerance	Hybrid breeding system (InVigor [®])	Antibiotic resistance
T45	AC EXCEL ¹	<i>pat</i>	-	-
Topas 19/2	Topas ¹	<i>pat</i>	-	<i>nptII</i>
MS1	Drakkar ²	<i>bar</i>	<i>barnase</i>	<i>nptII</i>
RF1 & RF2	Drakkar ²	<i>bar</i>	<i>barstar</i>	<i>nptII</i>
MS8*	Drakkar ²	<i>bar</i>	<i>barnase</i>	-
RF3*	Drakkar ²	<i>bar</i>	<i>barstar</i>	-

* Lines which Bayer seeks to commercialise in Australia 1- commercialised European variety 2 - commercialised Canadian variety

87. Both the barnase and barstar genes are derived from the soil bacterium *Bacillus amyloliquefaciens* (Harley 1989). The mechanism by which barnase and barstar genes confer male sterility or restoration of fertility are described in detail in Section 3.1.
88. The herbicide-tolerance trait in each of the GM canola lines is conferred by the presence of either the bar gene from the bacterium *Streptomyces hygroscopicus* or the pat gene from the bacterium *Streptomyces viridichromogenes*. Both the pat and bar genes encode the enzyme phosphinothricin acetyl transferase, which detoxifies phosphinothricin - the active constituent in the post-emergent, broad-spectrum herbicide glufosinate ammonium.
89. Glufosinate ammonium is not currently registered for use in broad-acre agriculture in Australia. Registration of herbicides is undertaken the Australian Pesticides and Veterinary Medicines Authority (, formerly the National Registration Authority for Agricultural and Veterinary Chemicals, NRA). Glufosinate ammonium is only registered in Australia as Basta[®] for horticultural uses and as Finale[®] for use in non-crop agricultural areas, commercial and industrial areas and rights-of-way. Bayer has been granted registration of glufosinate ammonium, under the trade name Liberty[®], for use on their glufosinate ammonium tolerant GM canola. The herbicide-tolerant phenotype can serve as a dominant marker for the introduced traits during breeding and hybrid seed production and may also be used for the control of weeds in the canola crop.
90. The antibiotic resistance trait in the GM canola lines Topas 19/2, RF1, RF2 and MS1 is conferred by the nptII gene from transposon Tn5 of *Escherichia coli*. The nptII gene encodes the enzyme neomycin phosphotransferase II (NPTII, also known as aminoglycoside 3'-phosphotransferase II, APH(3')II) which detoxifies aminoglycoside antibiotics such as kanamycin.
91. More details on each of the genes introduced to the GM canola lines are provided in Section 3 below. Bayer has presented data describing the molecular characterisation of the genetic modifications for all seven of the GM canola lines.

92. The methods used to introduce the genes into canola are discussed in Section 4 of this Appendix. An analysis of the potential for GM canola to be a weed and the potential for transfer of genes from the GM canola to other organisms, including weedy Brassicaceous relatives, is provided in Appendices 4 and 5 respectively.

SECTION 2 THE PARENT ORGANISM

93. A comprehensive review of the parent organism is provided in “Biology and Ecology of Canola (*Brassica napus*)” (OGTR 2002a), available from the OGTR website (www.ogtr.gov.au). Canola of cultivar Drakkar was transformed in the development of lines RF1, RF2, RF3, MS1 and MS8; AC EXCEL cultivar for T45; and cultivar Topas for Topas 19/2. Drakkar is a commercial Canadian canola cultivar and AC Excel and Topas are commercial European canola cultivars.

SECTION 3 THE INTRODUCED GENES

Section 3.1 The *barnase* gene

94. The male sterile lines of the GM canola, MS1 and MS8, were produced by genetically modifying the parental line by the introduction of the barnase gene from the bacterium *Bacillus amyloliquefaciens* (Canadian Food Inspection Agency 1995b; Canadian Food Inspection Agency 1996b; USDA-APHIS 1999b; USDA-APHIS 2002a).
95. The barnase gene encodes a ~12kD ribonuclease (RNase) called Barnase (Hartley 1988). RNases are enzymes that degrade ribonucleic acid (RNA), the biochemical intermediate between a gene (DNA) and the protein it encodes. RNases are ubiquitous in nature and serve many biological functions. The bacterium *B. amyloliquefaciens*, from which the gene is derived is a commonly occurring soil bacterium and is frequently used as a source for industrial enzymes such as alpha amylase (ANZFA 2001a).
96. The introduced barnase gene is under the control of an anther-specific promoter PTA29 derived from *Nicotiana tabacum* (Mariani et al. 1990; Seurinck et al. 1990) in both male sterile lines MS1 and MS8 (European Scientific Committee on Plants 1998b; Health Canada 1999a; USDA-APHIS 1999b; Health Canada 1999b; USDA-APHIS 2002a). (A promoter is a small piece of DNA that controls the level of expression of genes, acting like a switch). The mRNA polyadenylation signals, which are required for gene expression in plants, are provided by the 3' non-translated region of the nopaline synthase gene (3' nos) from *Agrobacterium tumefaciens* (Depicker et al. 1982; Dhaese et al. 1983; European Scientific Committee on Plants 1998b; USDA-APHIS 1999b; USDA-APHIS 2002a). Anther-specific expression of the barnase gene results in production of the cytotoxic RNase only in the tapetum cell layer of the pollen sac during anther development, destroying those cells, preventing pollen formation and resulting in male sterility (Mariani et al. 1990; De Block & De Bouwer 1993).

Section 3.2 The *barstar* gene

97. The fertility restorer lines of the GM canola, RF1, RF2 and RF3, were produced by genetically modifying the parental line by the introduction of the barstar gene from *B. amyloliquefaciens* (Hartley 1988; European Scientific Committee on Plants 1998b; USDA-APHIS 1999b; USDA-APHIS 2002a). The fertility restorer lines RF1 and RF2 were generated by the insertion of the same DNA construct. The barstar gene encodes a ~10kD ribonuclease inhibitor protein, Barstar, that binds specifically to the Barnase

- RNase, forming a stable complex that suppresses the ribonuclease activity (Hartley 1988; Hartley 1989).
98. The introduced barstar gene in GM canola lines RF1, RF2 and RF3 is under the control of the same regulatory sequences as the barnase gene in line MS1 and MS8 (European Scientific Committee on Plants 1998b; Health Canada 1999a; USDA-APHIS 1999b; Health Canada 1999b; USDA-APHIS 2002a): the anther-specific PTA29 promoter from *N. tabacum* (Seurinck et al. 1990; Mariani et al. 1992) and the mRNA polyadenylation signals from the 3' non-translated region of the nos gene from *A. tumefaciens* (Depicker et al. 1982). Anther-specific expression of the barstar gene results in production of Barstar protein only in the tapetum cell layer of the pollen sac during anther development (Mariani et al. 1992).
 99. Progeny resulting from pollination of a male sterile MS line by a fertility restorer RF line, eg MS1xRF1 or MS8xRF3, will express both the Barnase and Barstar proteins in the tapetum cells during anther development. The Barstar protein will bind to the Barnase protein, inactivating the cytotoxic RNase activity of the Barnase and thereby enabling the hybrid plants to develop normal anthers and pollen and thereby restore fertility (Mariani et al. 1992).
 100. The Barnase and Barstar proteins are also discussed in Appendices 2 and 3, toxicity and allergenicity risks of the GMOs.

Section 3.3 The *bar* and *pat* genes

101. Each of the genetically modified canola lines have been genetically modified for tolerance to the herbicide glufosinate ammonium by the introduction of either the bar gene from the bacterium *Streptomyces hygroscopicus* (Murakami et al. 1986; Thompson et al. 1987) or the pat gene from *Streptomyces viridochromogenes* (Wohlleben et al. 1988; Strauch et al. 1988). Both the pat and bar genes encode the enzyme phosphinothricin acetyl transferase (Wohlleben et al. 1988). *Streptomyces spp.* are saprophytic, soil-borne microbes and are not considered a pathogen of plants, humans, or other animals (Organisation for Economic Co-operation and Development (OECD) 1999b).
102. Glufosinate ammonium acts as a herbicide by inhibiting the plant enzyme glutamine synthetase, leading to ammonia accumulation, inhibition of amino acid synthesis and inhibition of photosynthesis, leading to severe damage to plant tissues, ultimately killing the plant (Pline 1999). Glufosinate ammonium is the active ingredient of a number of proprietary herbicides including Basta[®], Finale[®] (Australia) and Liberty[®] (other countries).
103. Glufosinate ammonium is made up of an equimolar, racemic mixture of the D- and L-isomers of phosphinothricin (PPT) and is registered for use as a herbicide in many countries (Organisation for Economic Co-operation and Development (OECD) 1999b). The terms glufosinate ammonium and phosphinothricin are often used synonymously.
104. PPT is the amino acid, 4-[hydroxy-(methyl) phosphinoyl]-D,L-homoalanine. L-PPT was initially characterised as a component of the tripeptide (phosphinothricyl-L-alanyl-L-alanine) antibiotic bialaphos (hence bar - bialaphos resistance) produced naturally by *S. hygroscopicus* and *S. viridochromogenes* (Organisation for Economic Co-operation and Development (OECD) 1999b). L-PPT was later shown to be effective as a broad-spectrum herbicide (Hoerlein 1994). The D-isomer, D-PPT, exhibits no herbicidal activity.

105. The PAT enzyme detoxifies glufosinate ammonium by acetylation of the L-isomer into N-acetyl-L-glufosinate ammonium (NAG) which does not inhibit glutamine synthetase (Droge-Laser et al. 1994; Organisation for Economic Co-operation and Development 2002) and therefore confers resistance to the herbicide (Organisation for Economic Co-operation and Development (OECD) 1999b; Organisation for Economic Co-operation and Development 2002). In the natural situation PAT prevents autotoxicity from bialaphos in *S. hygroscopicus* and *S. viridochromogenes* (Kumada et al. 1988).
106. The glufosinate ammonium tolerance trait was introduced into the GM canola lines as a selectable marker to identify transformed plants during tissue culture regeneration, as a dominant marker in breeding and hybrid seed production, and to enable the use of glufosinate ammonium as an alternative herbicide to control weeds in the canola crop. As mentioned above, Bayer has been granted registration of glufosinate ammonium as Liberty[®] by APVMA for use on InVigor[®] canola.
107. GM canola lines RF1, RF2, RF3, MS1 and MS8 contain the bar gene (Canadian Food Inspection Agency 1995b, 1996b; European Commission 1996; European Scientific Committee on Plants 1998b; USDA-APHIS 1999a, 1999b, 2002a, 2002c;) and lines T45 and Topas 19/2 contain the pat gene (Canadian Food Inspection Agency 1995a, 1996c; USDA-APHIS 1998a, 1998b, 2002b, 2002d;)
108. The bar and pat genes are very similar with an overall identity of 87% at the nucleotide sequence level, and both encoding PAT proteins of 183 amino acids with 85% at amino acid sequence identity, comparable molecular weights (~22kD) and similar substrate affinity and biochemical activity (Wehrmann et al. 1996).
109. The DNA sequence of both the pat and bar genes introduced into the GM canola lines was modified for plant-preferred codon usage to ensure optimal expression in *Brassica napus* (Health Canada 1997a; European Scientific Committee on Plants 1998a, 1998b; USDA-APHIS 1999b). Amino acids, the building blocks of proteins, are encoded in DNA by nucleotide triplets called codons. Some amino acids may be encoded by up to six different codons and the 'bias' of which codon is most frequently used varies between organisms, with plants having a different bias than bacteria).
110. The PAT protein produced from the pat gene in GM canola lines T45 and Topas 19/2 has exactly the same amino acid sequence as the native protein from *S. viridochromogenes*.
111. The bar gene introduced into GM canola lines MS8 and RF3 was modified by the substitution of the N-terminal two codons of the bacterial gene, GTG and AGC (Thompson et al. 1987), with the codons ATG and GAC (USDA-APHIS 1999b).
112. Expression of the bar gene in the GM canola lines RF1, RF2, RF3, MS1 and MS8 is controlled by the plant promoter PSsuAra from the S1A ribulose-1,5-bisphosphate carboxylase (Rubisco) small subunit gene from the plant *Arabidopsis thaliana* (Krebbers et al. 1988; European Scientific Committee on Plants 1998b). The PSsuAra promoter directs gene expression in green plant tissues (Krebbers et al. 1988).
113. The mRNA polyadenylation signals for the bar gene in GM canola lines RF1, RF2, RF3, MS1 and MS8 are derived from the 3' non-translated region from the T-DNA gene 7 (3'g7) of *A. tumefaciens* (Dhaese et al. 1983; Velten & Schell 1985; European Scientific Committee on Plants 1998b; USDA-APHIS 2002a).
114. In lines RF1, RF2 and MS1, post-translational targeting of the bar gene product (PAT) to the chloroplast is accomplished by fusion of the 5' terminal coding sequence

of bar with the chloroplast transit peptide coding sequence of the S1A Rubisco gene from *A. thaliana* (Krebbers et al. 1988; Health Canada 1999a, 1999b; ANZFA 2001a; USDA-APHIS 2002a, 2002c).

115. A transit peptide is a stretch of amino acids on the amino-terminal portion of a protein that targets the protein to a specific organelle and is removed during or immediately after transport into the organelle. It has been shown that the chloroplast transit peptides are rapidly degraded *in vivo* after cleavage by cellular proteases (Bartlett et al. 1982; della-Cioppa et al. 1986). Chloroplasts contain significant levels of glutamine synthetase, the target of glufosinate ammonium inhibition (Cren & Hirel 1999).
116. Except for the first two amino acids, the PAT protein produced from the bar gene in GM canola lines RF1, RF2, RF3, MS1 and MS8 has the same amino acid sequence as the native protein from *S. hygroscopicus*.
117. The pat gene is controlled by the constitutive 35S promoter and 35S mRNA polyadenylation signals from cauliflower mosaic virus (CaMV, Odell et al. 1985) in both lines T45 (USDA-APHIS 1998a, 1998b) and Topas 19/2 (European Scientific Committee on Plants 1998a; USDA-APHIS 2002d).

Section 3.4 The *nptII* gene

118. An antibiotic resistance gene, *nptII*, has been transferred into lines Topas 19/2, MS1, RF1 and RF2. The *nptII* gene is derived from transposon Tn5 from the bacterium *E. coli* (Beck et al. 1982) and codes for the ~29kD enzyme neomycin phosphotransferase (NPTII) conferring resistance to aminoglycoside antibiotics such as kanamycin and neomycin. The *nptII* gene functioned as a selectable marker in the initial laboratory stages of development of the GM plants, enabling selection of plant cells containing the desired genetic modification following transformation (Fraley et al. 1983; De Block et al. 1984).
119. The NPTII enzyme catalyzes the transfer of a phosphate group from adenosine 5'-triphosphate (ATP) to a hydroxyl group of aminoglycoside antibiotics as well as butirosins, thereby inactivating the antibiotics (Davies and Smith 1978; Goldman and Northrop 1976).
120. Of the antibiotics that are inactivated by the NPTII enzyme, ribostamycin, kanamycin, and paromomycin are not registered for use in Australia. Only neomycin and gentamicin are currently in therapeutic use for humans or animals. Both are infrequently used, neither is unique for any use, and oral administration of either of them is rare (<http://vm.cfsan.fda.gov/~dms/opa-armg.html>; Commonwealth Department of Health and Aged Care 1998; National Registration Authority 2002). Furthermore, the gentamicin that is used for human therapeutic use is composed primarily of gentamicin C₁ (25-50%), gentamicin C_{1a} (10-35%), and gentamicins C_{2a} and C₂ (25-55%) (David Bull Laboratories pers. comm. Oct. 2002). NPTII inactivates only gentamicins A and B and therefore does not confer resistance to the gentamicin that is used therapeutically.
121. Expression of the *nptII* gene is controlled by the nopaline synthase promoter (P-nos) from *A. tumefaciens* (Bevan et al. 1983) and the mRNA polyadenylation signals derived from the 3' non-translated region of the octopine synthase gene (3' ocs) from *A. tumefaciens* (Dhaese et al. 1983) in GM canola lines Topas 19/2 (European Scientific Committee on Plants 1998a; USDA-APHIS 2002d), RF1, RF2 and MS1 (USDA-APHIS 2002a).

122. It is important to note that the *nptII* gene is common in the environment, occurring naturally in bacteria in soil, water and in the intestinal tract of humans and animals.

Section 3.5 Regulatory sequences

123. Although some of the regulatory sequences controlling the introduced genes in the various GM lines are derived from the plant pathogens *A. tumefaciens* and cauliflower mosaic virus, these sequences cannot induce disease. The various regulatory sequences controlling the expression of the introduced genes in the GM canola lines are summarised in Table 2.

Table 2: Genetic elements and their origin

Gene	Promoter	3' transcription termination and polyadenylation signals
<i>bar</i> <i>Streptomyces hygroscopicus</i>	PSsuAra <i>Arabidopsis thaliana</i> expressed in green tissues	3'g7 <i>Agrobacterium tumefaciens</i>
<i>pat</i> <i>Streptomyces viridochromogenes</i>	P-35S <i>Cauliflower mosaic virus</i> Constitutive promoter	T-35S Cauliflower mosaic virus
<i>barnase and barstar</i> <i>Bacillus amyloliquifaciens</i>	PTA29 <i>Nicotiana tabacum</i> tapetum- specific promoter	3' nos <i>Agrobacterium tumefaciens</i>
<i>nptII</i> Tn5 of <i>Escherichia coli</i>	P-nos <i>Agrobacterium Tumefaciens</i> weak constitutive promoter	3'ocs <i>Agrobacterium tumefaciens</i>

SECTION 4 METHOD OF GENE TRANSFER

124. All seven of the GM canola lines were produced using a disarmed *Agrobacterium tumefaciens* - mediated transformation system to introduce the genes of interest (De Block et al. 1989).
125. The *Agrobacterium*-mediated DNA transformation system is well understood (Zambryski 1992). The plasmid vector used in transformation to produce line Topas 19/2 was a binary transformation vector. The plasmid vectors used for the other six lines were co-integrative (information supplied by Bayer). The plasmids contain well characterised DNA segments required for selection and replication of the plasmid in bacteria as well as the sequences essential for DNA transfer from *Agrobacterium* and integration in the plant cell genome (Bevan 1984; Wang et al. 1984)
126. *A. tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants. The molecular biology of crown gall disease shows that plants can be genetically transformed by the transfer of DNA (T-DNA, located between specific border sequences) from *A. tumefaciens* through the mediation of the genes (*vir* region) of Ti plasmids. The specific border sequences are known as the left and right borders (LB and RB) and these delimit the DNA to be transferred. Disarmed *Agrobacterium* strains have been constructed specifically for plant transformation. The disarmed strains cannot cause crown gall disease because they do not contain the genes (*iaaM*, *iaaH* and *ipt*) for the overproduction of auxin and cytokinin, which are required for tumour induction and rapid callus growth (Klee & Rogers 1989). A useful feature of the Ti plasmid is the flexibility of the *vir* (*virulence*) region to act in either *cis* or *trans* configurations to the T-DNA. This has allowed the development of two types of

- transformation systems (both of which provide functionally equivalent transformation systems):
- co-integration vectors that join the T-DNA that is to be inserted into the plant and the *vir* region in a single plasmid (Stachel & Nester 1986);
 - binary vectors that have the T-DNA and *vir* regions segregated on two plasmids (Bevan 1984).
127. The plasmid vectors used to generate the GM canola lines T45, RF1, RF2, MS1, RF3 and MS8 contain the Sm/Sp gene, which confers resistance to the aminoglycoside antibiotics streptomycin and spectinomycin. Topas 19/2 contains a gene that confers resistance to the antibiotic tetracycline. The antibiotic resistance genes are used as markers that enable selection of bacteria containing the plasmid. These marker genes are located outside the left and right border sequences of the T-DNA that delimit the DNA to be transferred and were not transferred into any of the GM canola lines. The absence of the vector sequences in the plants was confirmed by Southern blot and DNA sequence analysis (data supplied by Bayer).
128. Sequences outside the T-DNA borders (which were not transferred) for each of the plasmids used to generate the seven GM canola lines contained:
- the ColE1 origin of replication from pBR322 for replication in *E. coli*;
 - the pVS1 origin of replication from *Pseudomonas* plasmid pVS1 for replication in *Agrobacterium tumefaciens*; and
 - a gene conferring resistance either to the antibiotics streptomycin and spectinomycin, or tetracycline for propagation and selection of the plasmids in *E. coli* and *A. tumefaciens*.
129. It should be noted that some detailed technical information on precise gene constructs, including maps of the plasmids used to generate GM canola lines Topas 19/2, RF1 and RF2, and MS1 has been declared as Confidential Commercial Information (CCI) under Section 185 of the Act. The same plasmid construct was used to generate both lines RF1 and RF2.
130. The details of the plasmids used to generate GM canola lines T45, MS8 and RF3 - pHOE4/Ac(II), pTHW107 and pTHW118 respectively, have previously been publicly described (FDA 1997; European Scientific Committee on Plants 1998b; De Both & De Beuckeleer 2001; APEC 2001; OGTR 2002g). The backbone sequence of pHOE4/Ac(II) was derived from the plant transformation vector pPCV002 (Koncz & Schell 1986). The backbone sequences of plasmids pTHW107 and pTHW118 were derived from pGSV1, which was derived from pGSC1700 (Cornelissen & Vandewiele 1989).
131. The pHOE4/Ac(II) plasmid used to generate GM canola line T45 contains the following elements between the left and right borders:
- the 35S promoter from cauliflower mosaic virus (CaMV);
 - the *pat* gene from *S. viridichromogenes*; and
 - the 35S terminator from cauliflower mosaic virus (CaMV).
132. The pTHW107 plasmid used to generate the male sterile canola line MS8 contains the following elements between the left and right borders:
- the PTA29 promoter from *N. tabacum*;
 - the *barnase* gene from *B. amyloliquefaciens*;
 - the 3' untranslated region of the *nos* gene from *A. tumefaciens*;

- the PSsuAra promoter from *A. thaliana*;
 - the *bar* gene from *S. hygrosopicus*;
 - the 3' untranslated region of *gene 7* from *A. tumefaciens*.
133. The pTHW118 plasmid used to generate the male sterile canola line Rf3 contains between the left and right borders:
- the PTA29 promoter from *N. tabacum*;
 - the *barstar* gene from *B. amyloliquefaciens*;
 - the 3' untranslated region of the *nos* gene from *A. tumefaciens*;
 - the PSsuAra promoter from *A. thaliana*;
 - the *bar* gene from *S. hygrosopicus*;
 - the 3' untranslated region of *gene 7* from *A. tumefaciens*.
134. In Topas 19/2, the T-DNA also contains a cos site of bacteriophage lambda, a ColE1 origin of replication from *E. coli*, and a supF suppressor tRNA gene from *E. coli* (data supplied by Bayer, European Scientific Committee on Plants 1998a). These genetic elements are of non-eukaryotic origin and will not function in the plant. The origin of replication and cos sequences are not coding sequences. The supF tRNA gene encodes a transfer RNA (tRNA), however its expression is under the control of prokaryotic promoter that will not be recognised in plants. Even if the supF tRNA gene could be expressed in the plant, the resultant tRNA would not function because of the differences between the translational machinery of prokaryotes and eukaryotes.

SECTION 5 CHARACTERISATION OF THE INSERTED GENETIC MATERIAL AND STABILITY OF THE GENETIC MODIFICATION

135. The genetic modification in each of the seven GM canola lines has been characterised at the molecular level, including determination of the site of insertion by DNA sequencing. A summary of the molecular characterisation of these lines is given in Table 3.

Section 5.1 Male sterile GM canola line MS8

136. Southern blot analysis was used to demonstrate that a single insertion event had occurred in GM canola line MS8 when transformed with plasmid pTHW107. Further analysis utilising the polymerase chain reaction (PCR) technique, and molecular cloning and sequencing of the site of insertion revealed that, as intended, only one T-DNA insert containing full-length copies of the barnase and bar genes had been integrated into the genome of line MS8 (data supplied by Bayer).
137. Southern blot analysis of progeny of three generations of GM canola line MS8 (T1, T3 and Backcross 1) indicates that the inserted DNA is stably integrated and inherited (data supplied by Bayer). Experience from previous releases of line MS8 in Australia over five years has shown that the male sterile and glufosinate ammonium-tolerance traits have been stably inherited over multiple generations. The traits exhibit Mendelian inheritance, functioning as dominant genes.

Section 5.2 Fertility restorer GM canola line RF3

138. Southern blot analysis was used to demonstrate that a single insertion event had occurred in GM canola line RF3 from transformation with pTHW118. Further analysis utilising the polymerase chain reaction (PCR) technique, and molecular cloning and sequencing of the site of insertion revealed that the DNA integrated into the genome of line RF3 comprises a complete T-DNA copy arranged in an inverted repeat configuration with a second, incomplete T-DNA copy of the T-DNA (data supplied by Bayer, European Scientific Committee on Plants 1998b; USDA-APHIS 1999b).
139. The partial copy of the T-DNA included a truncated but functional pTA29 promoter, a complete copy of the barstar gene and the 3' nos sequence, and a truncated, non-functional portion of the PSsuAra promoter (data supplied by Bayer, European Scientific Committee on Plants 1998b). Analysis of the site of insertion also revealed that there was a duplication of plant DNA on either side of the insert (data supplied by Bayer).
140. The plant genomic DNA sequences flanking the left and right borders at the site of insertion were analysed for possible homology to known genes by comparison with the GenBank, EMBL, DDBJ, PDB sequence databases using the BLAST algorithm (Altschul et al. 1990). No significant homology to known genes was identified.
141. Southern blot analysis of progeny of three generations of GM canola line RF3 (S1, S3 and Backcross 1) indicates that the inserted DNA is stably integrated and inherited (data supplied by Bayer). The applicant has indicated that experience from previous releases of line RF3 in Australia over five years has shown that the fertility restorer and glufosinate ammonium-tolerance traits have been stably inherited over multiple generations. The traits exhibit Mendelian inheritance, functioning as dominant genes.

Section 5.3 Fertility restorer lines RF1, RF2, MS1, and glufosinate ammonium tolerant lines T45 and Topas 19/2

142. Southern blot and segregation analyses of progeny of GM canola lines T45, Topas 19/2, RF1, RF2 and MS1 were used to demonstrate that a single insertion event has occurred in each of these lines and that the inserted DNA is stably integrated and inherited (data supplied by Bayer). Only a single copy of the T-DNA was inserted in lines T45, RF1, RF2 and MS1. Experience from previous releases of these lines in Australia and overseas also demonstrates that the herbicide tolerance (all lines), male sterility (MS1) and fertility restoration (RF1, RF2) traits are stably inherited over multiple generations and that the traits exhibit Mendelian inheritance, functioning as dominant genes.
143. In line Topas 19/2, there is a single insertion event that has resulted in a head to head inverted repeat of the T-DNA, such that there are two complete copies of each of the inserted genes pat and nptII (data supplied by Bayer, FDA 1995; European Scientific Committee on Plants 1998a)
144. In addition, the DNA sequence of the inserted DNA between the left and right borders was determined for lines T45 and MS1, confirming that the T-DNA integrated into the plant was identical to that in the plasmid used for transformation, and further confirming that no sequence rearrangements had occurred in these lines.
145. The plant genomic DNA flanking the left and right borders at the site of integration was characterised by DNA sequencing for all five lines. The flanking sequences were analysed for possible homology to known genes by comparison with genes in DNA sequence databases using the BLAST algorithm.

146. No significant homology to known genes was identified for the flanking regions of lines T45 or Topas 19/2. No significant homology to known genes was identified for the left border flanking regions of line MS1 and RF1, or for the right border flanking region of line RF2.
147. Significant homology was detected for the right border flanking regions of line MS1, RF1 and for the left border flanking region of line RF2 to *Arabidopsis thaliana*. However, in each case the homology was not to any genes with a known function. The entire genome of *A. thaliana* has recently been sequenced (The Arabidopsis Genome Initiative 2000). *A. thaliana* and *B. napus* both belong to the 'Family Brassicaceae' and molecular genetic studies indicate that they have a close evolutionary relationship (Cavell et al. 1998; Parkin et al. 2002). The observed sequence homology is therefore not surprising.

Table 3 Molecular Characterization of GM canola lines

LINE	GENE	Transgene Integration			
		Number of gene copies	Stable integration and inheritance	Inserted genes verified by DNA sequence	Flanking regions determined by DNA sequence
MS8	<i>bar</i>	1	Yes	Yes	Yes
	<i>barnase</i>				
RF3	<i>bar</i>	1	Yes	Yes	Yes
	<i>barstar</i>	2			
MS1	<i>bar</i>	1	Yes	Yes	Yes
	<i>npIII</i>				
	<i>barnase</i>				
RF1 & RF2	<i>bar</i>	1	Yes	Yes	Yes
	<i>npIII</i>				
	<i>barstar</i>				
T45	<i>pat</i>	1	Yes	Yes	Yes
Topas19/2	<i>pat</i>	2	Yes	Yes	Yes
	<i>npIII</i>				

SECTION 6 EXPRESSION OF THE INTRODUCED GENES

148. The level of expression of the introduced proteins in each of the seven GM canola lines has been assessed in a variety of ways: phenotype, mRNA expression, enzyme activity and detection of the protein by ELISA or Western blotting.

Section 6.1 Phosphinothricin acetyltransferase

149. All seven of the GM canola lines contain either the *bar* or the *pat* gene encoding the PAT protein. The expression of the PAT protein in each of the GM canola lines is demonstrated by the herbicide-tolerant phenotype – the plants survive the application of the herbicide glufosinate ammonium.
150. PAT expression in leaves of lines RF1 and MS1, and RF2, and in leaves and seeds of lines RF3 and MS8, was investigated in separate experiments by PAT enzyme activity. The level of PAT activity in leaves in lines RF3 and MS8 was greater than in seeds. The level of PAT in leaves of MS and RF lines were comparable. The results of these experiments are summarised in Table 4.

Table 4 PAT expression in seeds and leaves of lines RF3 and MS8 determined by enzyme activity

GM canola line	Seed µg/mg total protein	Leaf µg/mg total protein
RF3	0.10	1.33
MS8	0.04	0.51
RF1	Not tested	1.45
RF2	Not tested	0.7
MS1	Not tested	0.9

151. PAT expression in seeds of lines T45, Topas 19/2, RF1, RF2, RF3, MS1 and MS8 was investigated in a series of separate experiments by enzyme linked immunosorbent assay (ELISA). The levels of PAT protein detected in these experiments were very low – of the order of 8 ng/mg total protein or 0.0008% of total protein. Similarly the level in seed on a per gram seed basis is very low. These results are summarised in Tables 5 and 6.

Table 5 PAT expression in seeds of GM canola lines determined by ELISA

GM canola line	µg PAT/g seed	µg PAT/mg total protein
RF3	0.69	0.012
MS8	0.07	0.002
RF3xMS8	0.34	0.013
RF1	0.50	0.015
RF2	0.42	0.012
MS1	0.07	0.002
MS1xRF1	0.20	0.006
MS1xRF2	0.35	0.007

Table 6 PAT expression in seeds of GM canola lines determined by ELISA

GM canola line	Seed µg PAT/g seed	Leaf µg PAT/g fresh weight
T45	0.561	0.348
Topas	0.47	0.0843

152. The level of PAT in oil and meal derived from processing of seed from lines T45 and Topas 19/2 was investigated by ELISA. No PAT protein was detected in canola oil derived from the GM canola lines. While PAT protein could be detected by ELISA at less than 0.005% of total protein in toasted canola meal, the processing of canola seed to produce edible oil and meal for animal feed denatures the PAT protein and destroys the enzymatic activity (FDA 1995; FDA 1997; European Scientific Committee on Plants 1998a; European Scientific Committee on Plants 1998b; ANZFA 2001a).

153. Expression of the bar gene was also investigated by Northern analysis, with mRNA being detected in leaves and flower buds of lines RF1, RF2 (data supplied by Bayer) and RF3 and MS8 (European Scientific Committee on Plants 1998), with greater abundance in leaves than flower buds. In line MS1 bar mRNA was detected in leaves but not in flower buds. The presence of bar mRNA in pollen in lines RF1, RF2 and MS1 was tested for but not detected (data supplied by Bayer). No bar mRNA was detected in dry seed from any of the lines RF1, RF2, RF3, MS1 or MS8 (data supplied

by Bayer) and these data are consistent with the very low levels of PAT protein detected in seed (European Scientific Committee on Plants 1998b; Health Canada 1999a, 1999b, ANZFA 2001a)

154. The levels of PAT protein and bar mRNA in the RF and MS lines are consistent with the fact that the bar gene is under the control of the PSsuAra promoter, which is expressed predominantly in green tissues (De Almeida et al. 1989)

Section 6.2 Barnase

155. The expression of the barnase gene in GM canola lines MS1 and MS8 was confirmed by the antherless, male sterile phenotype.
156. The expression of the barnase gene in lines MS1 and MS8 was also investigated by Northern analysis. No barnase mRNA was detected in flower buds, pollen, leaves or dry seed of lines MS1 or MS8 (European Scientific Committee on Plants 1998b; Health Canada 1999a; ANZFA 2001a). The cytotoxic expression of the barnase gene is very specific to the tapetum cell layer because of the PTA29 promoter (De Almeida et al. 1989; Mariani et al. 1990; Koltunow et al. 1990; De Block & De Bouwer 1993), with cell death occurring without accumulation of detectable levels of barnase mRNA (European Scientific Committee on Plants 1998b). Expression would not be expected in other tissues because of the specificity of the PTA29 promoter.

Section 6.3 Barstar

157. The expression of the barstar gene in GM canola lines RF1, RF2 and RF3 was confirmed by the phenotype of the progeny of RFxMS crosses - the plants are fully fertile with normal anther development as a result of the inhibition of the RNase activity of the Barnase protein by the Barstar inhibitor protein..
158. The expression of the barstar gene in GM canola line RF1, RF2 and RF3 was also investigated by Northern analysis and as predicted from the specificity of the PTA29 promoter, barstar mRNA was detected in flower buds but not in leaves, pollen or dry seed (European Scientific Committee on Plants 1998b; Health Canada 1999a; ANZFA 2001a).

Section 6.4 NPTII

159. The nptII gene is only present in GM canola lines Topas 19/2, RF1, RF2 and MS1.
160. The NPTII protein was detected in roots, leaves and buds but was not detected in seed in line Topas 19/2 as determined by ELISA (data supplied by Bayer, Canadian Food Inspection Agency 1995a; USDA-APHIS 2002d). Expression of NPTII was not detected in seed (data supplied by Bayer, USDA-APHIS 2002d). The Canadian Food Inspection Agency previously reported low levels of expression of NPTII in seed (Canadian Food Inspection Agency 1995a), however the applicant has advised that values recorded in these analyses were below the limit of detection of the ELISA test used.
161. The NPTII protein was not detected in seed in hybrid plants of RF1xMS1 crosses or RF2xMS1 crosses as determined by ELISA (data supplied by Bayer). NPTII protein was detected at very low levels in leaves of RF1, RF2 and MS1 plants as determined by non-denaturing gel electrophoresis and detection of enzymatic in situ phosphorylation of kanamycin with (γ -³²P) ATP (Reiss et al. 1984).

162. The expression of the nptII gene in lines RF1, RF2 and MS1 was also investigated by Northern analysis. No nptII mRNA was detected in flower buds, pollen, leaves or dry seed of lines RF1, RF2 or MS1 (FDA 1996a; ANZFA 2001a).
163. These low levels of nptII gene expression are consistent with the fact that nptII gene in GM canola lines Topas 19/2, RF1, RF2 and MS1 is under the control of P-nos, which is considered to be a constitutive, but weak promoter (Sanders et al. 1987; Harpster et al. 1988).
164. As mentioned above, the nptII gene occurs naturally in soil bacteria, water and in the intestinal tract of humans and animals.

Section 6.5 Other expression analyses

165. The possibility that the insertional event resulted in cryptic gene expression was investigated in lines RF1, RF2, RF3, MS1 and MS8. So called cryptic gene expression may result from the activation of normally silent (cryptic) promoter elements, as the result of integration of foreign DNA. These cryptic promoters are non-coding DNA sequences that are inactive under native conditions. Examples of such cryptic gene expression resulting from integration of foreign DNA have been described in plants (Fobert et al. 1994; Puzio et al. 1999). Northern blot experiments failed to detect any cryptic RNA expression of endogenous plant sequences or of the inserted T-DNA (data supplied by Bayer, European Scientific Committee on Plants 1998b).

CONCLUSION

166. The expression of each of the introduced genes in each of the GM canola lines Topas 19/2, T45, RF1, RF2, RF3, MS1 and MS8 has been determined by plant phenotype, mRNA expression, and detection of the novel protein by enzyme activity or ELISA. The patterns and levels of expression of the introduced proteins in the GM canola lines were as predicted on the basis of the promoters controlling expression, and a summary of these data is given in Table 7.
167. The PAT protein, which confers tolerance to the herbicide glufosinate ammonium, is expressed in all seven lines, with low levels in leaves and barely detectable levels in seed. The Barnase and Barstar proteins are expressed only in the developing anthers of flowers of MS and RF lines respectively, and in MSxRF hybrids. The NPTII protein is expressed only in lines Topas 19/2, RF1, RF2 and MS1, and is detected at very low levels in leaves, but not in seed. Only hybrid plants derived from crosses of lines RF1 or RF2 with line MS1 would express all four of the novel proteins.

Table 7 Summary of expression of the introduced proteins in GM canola

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

APPENDIX 2 HUMAN HEALTH AND SAFETY

168. Under section 51 of the Gene Technology Act 2000, the Regulator is required to consider risks to human health and safety or the environment in preparing the risk assessment and risk management plan. This part of the document considers potential hazards that may be posed to human health and safety. In this context, the potential toxicity and allergenicity of the GMOs or their novel proteins were considered.

SECTION 1 NATURE OF THE POTENTIAL TOXICITY OR ALLERGENICITY HAZARD

169. Toxicity is the cascade of reactions resulting from exposure to a dose of chemical sufficient to cause direct cellular or tissue injury or otherwise inhibit normal physiological processes (Felsot 2000b). Allergic responses are immune system reactions, resulting from stimulation of a specific group of antibodies (known as IgE) or sensitisation of specific tissue bound lymphocytes (Taylor & Lehrer 1996; FAO/WHO 2000). Allergy has a well defined etiology (ie. biochemical cause) that is quite different from toxicity.
170. The GM canola lines T45, Topas 19/2, RF1, RF2, RF3, MS1 and MS8, differ from conventional canola in the expression of four additional proteins, PAT, Barstar, Barnase and NPTII proteins. The potential of canola expressing these proteins to be toxic or allergenic to humans has been considered in detail in this Appendix. This could occur if the genetically modified canola were toxic or allergenic because of the novel gene products expressed in the plants, or if there were unforeseen, unintended effects of the genetic modification.
171. If the genetically modified canola was toxic or allergenic, there could be impacts relating to:
- safety of human foods containing canola oil (for example confectionery products, margarine, salad and cooking oil, mayonnaise, sandwich spreads, creamers and coffee whiteners). Responsibility for the assessment of the safety of food for human consumption lies with the Food Standards Australia New Zealand (FSANZ, formerly the Australia New Zealand Food Authority, ANZFA), not the Gene Technology Regulator. However, in accordance with the *Act*, the Regulator seeks advice from FSANZ on all applications and risk assessment and risk management plans. It should be noted that oil derived from all seven GM canola lines has been approved by FSANZ for human consumption (ANZFA 2001a).
 - occupational health and safety (for example, for farm workers, or factory workers involved in canola processing); and
 - environmental exposure (for example, people breathing canola pollen).
172. Canola has become more important to the western world as a foodstuff as a result of breeding for better oil quality and improved processing techniques (Organisation for Economic Co-operation and Development (OECD) 1997b). Unimproved varieties of *B. napus* tend to have high levels of the toxic compounds eucic acid and glucosinolates.
173. Oil suitable for human consumption was first extracted from the lines developed in Canada in 1956 (Colton & Potter 1999). Canola is now grown primarily for its seeds, which yield between 35% to over 45% w/w of edible oil. Cooking oil is the main use

but it is also commonly used in margarine. After oil is extracted from the seed, the remaining by-product, canola seed meal, is used as a high protein animal feed.

SECTION 2 LIKELIHOOD OF THE TOXICITY OR ALLERGENICITY HAZARD OCCURRING

Section 2.1 Toxicity

174. When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Sjoblad et al. 1992).
175. Apart from the introduced proteins, the toxicity hazard could occur if there were an unintended effect on the plant's metabolism, particularly if this affected erucic acid and glucosinolate levels.
176. In assessing the likelihood of adverse impacts due to toxicity of the seven GM canola lines, a number of factors were considered, including:
- toxicity of conventional canola;
 - toxicity of the new proteins expressed (PAT, Barnase, Barstar, NPTII);
 - changes to the levels of naturally occurring toxicants and nutritional factors; and
 - potential for altered metabolism of the herbicide.
177. This appendix presents data and conclusions from:
- acute oral toxicity studies in animals, which provide evidence about the toxicity of the four introduced proteins;
 - feeding studies with whole seeds in animals. As the feeding studies were conducted with whole seeds, these findings can also be used to investigate any unintended changes to the plant's metabolism;
 - compositional studies, which compare fatty acid levels (including erucic acid) in oil, and protein and glucosinolate levels in seed and meal, and compares these to levels in conventional varieties; and
 - an analysis of the toxicity of glufosinate ammonium herbicide metabolites.

2.1.1 Toxicity of conventional canola

178. Canola seed naturally contains the toxicants erucic acid and glucosinolates and it is important to determine if the levels of these known toxicants are altered in any of the seven GM canola lines. However, the term 'canola' refers to those varieties of *B. napus* that meet specific standards on the levels of erucic acid (C22:1 fatty acid) and glucosinolates. These cultivars must yield oil low in erucic acid (below 2% of the total fatty acids), (CODEX 2001) and meal low in glucosinolates (total glucosinolates of 30 μ moles/g toasted oil free meal) (Organisation for Economic Co-operation and Development (OECD) 2001), and are often referred to as 'double low' varieties.
179. Canola oil is the only fraction used for human food. As a quality control measure, no protein is allowed to be present in canola oil, therefore, oil derived from the GM canola lines would not contain any of the novel proteins. Oil derived from all seven GM canola lines has been approved for use in human food in Australia (ANZFA 2001a) and other countries (refer to Appendix 1).

180. The international standard for canola seed is that it must contain less than 2% erucic acid (CODEX 2001) and less than total glucosinolates of 30 µmoles of glucosinolates per gram of toasted, oil-free meal (Organisation for Economic Co-operation and Development (OECD) 2001). Further details can be obtained from the OGTR document on the biology of canola (OGTR 2002a).

2.1.2 Toxicity of the introduced proteins

181. Four novel proteins are expressed in the seven GM canola lines – phosphinothricin acetyl transferase (PAT), Barnase, Barstar and the neomycin phosphotransferase II (NPTII). However, not all of the novel proteins are expressed in all of the lines. Table 1 summarises which proteins are expressed in which GM canola lines (Refer to Appendix 1 for more details on the genes that code for these proteins and their origin).

Table 1 Novel proteins expressed in GM canola lines

	PAT	Barstar	Barnase	NPTII
RF3*	Yes	Yes	No	No
MS8*	Yes	No	Yes	No
RF3 x MS8*	Yes	Yes	Yes	No
RF1, RF2	Yes	Yes	No	Yes
MS1	Yes	No	Yes	Yes
RF1 x MS1, RF2 x MS1	Yes	Yes	Yes	Yes
T45	Yes	No	No	No
Topas 19/2	Yes	No	No	Yes

*RF3 and MS8, and hybrids derived from them, are the lines which Bayer proposes to commercialise in Australia.

182. No sequence homology has been found between the PAT, NPTII, Barstar or Barnase proteins and known toxins in various sequence databases (Van den Bulcke 1997; data supplied by Bayer, ANZFA 2001a).
183. All seven GM canola lines express the PAT protein (either through the pat gene or the bar gene) that confers tolerance to the herbicide glufosinate ammonium. Only the MS and RF lines express the Barnase and Barstar proteins respectively, and MS x RF hybrids will express both proteins. Only lines MS1, RF1, RF2 and Topas 19/2 express the NPTII protein.
184. The intended lines for commercial release, MS8 and RF3 and their hybrid RF3 x MS8 hybrid canola will express the PAT, Barstar and Barnase proteins.
185. Each of the introduced proteins is expressed in low levels (Appendix 1). All of the genes that encode these proteins are derived from commonly occurring bacteria – *Streptomyces hygroscopicus*, *Streptomyces viridochromogenes*, *Bacillus amyloliquefaciens* and *Escherichia coli*. *Streptomyces* species and soil actinomycetes are not implicated in disease. *Bacillus amyloliquefaciens* is used in the food industry (ANZFA 2001a). *E. coli* is a commensal bacterium of the human gut.
186. Each of the GM canola lines included in this application has been assessed by several other regulatory agencies with regard to toxicity and allergenicity whose recommendations are also noted (Canadian Food Inspection Agency 1995a; Canadian Food Inspection Agency 1995b; European Commission 1996; FDA 1996a; Canadian Food Inspection Agency 1996b; Canadian Food Inspection Agency 1996c; Canadian Food Inspection Agency 1996d; FDA 1997; Health Canada 1997a; Bjerregaard 1998; USDA-APHIS 1998a; USDA-APHIS 1998b; European Scientific Committee on Plants

1998b; Health Canada 1999a; USDA-APHIS 1999a; Health Canada 2000; USDA-APHIS 2002a; USDA-APHIS 2002b; USDA-APHIS 2002c).

2.1.2.1 PAT protein

187. All of the seven GM canola lines express the PAT protein, encoded by either the bar or the pat gene. PAT is responsible for detoxifying phosphinothricin (glufosinate ammonium).
188. There is no evidence that the PAT protein is toxic to either humans or other animals. The potential for PAT to be toxic has been addressed via acute toxicity studies.
189. In a 14-day acute toxicity study, mice fed with high levels of the recombinant PAT protein (2,500 milligram/kilogram bodyweight) showed no treatment-related significant toxic effects (Merriman 1996). In this study, 10 mice (five male and five female) were administered a single dose of the His-tag PAT /kg body weight. A His-tag is a stretch of amino acid (Histidine) residues attached to the protein molecule that aid in its purification by columns that bind Histidine. Body weights of the test animals were determined prior to dosing (day 0), and on days 7 and 14 after dosing and the animals were observed daily for any clinical abnormalities or mortality. No mortality occurred during the study. Following scheduled euthanasia of test animals on day 14, no gross internal findings were observed. Based on this test, the acute oral LD50 was estimated to be greater than 2500 mg of His-tag PAT/kg body weight.
190. In addition, a study by Pfister et al (1996, cited in Bremmer & Leist 1996) also investigated the toxicity of purified PAT protein in a repeated dose oral toxicity study in rats. Groups of five male and five female rats were fed PAT protein for 14 days at levels of 0, 0.5 or 5% of their diet (equivalent to 0, 707 and 7792 mg/kg body weight/day). The highest concentration is 6 million times the PAT concentration in GM canola grain. No adverse effects or mortality were observed during the study, even at the highest dose of the PAT protein. At day 14 the rats were euthanized and the following parameters were investigated at necropsy:
 - total and differential white blood count;
 - spleen and thymus weight; and
 - histological examination of spleen, thymus, mesenteric lymph node, Peyer's patches and bone marrow.
191. No significant differences were observed for any of these parameters upon necropsy - even at the highest dose of PAT protein. Based on this study, the LD50 of PAT was estimated to be greater than 7792 mg/kg body weight.
192. The lack of toxicity of the PAT protein expressed in genetically modified plants has been assessed by a number of regulatory bodies in Australia, USA, Canada, and Europe (FDA 1995; FDA 1997; European Scientific Committee on Plants 1998a; European Scientific Committee on Plants 1998b; ANZFA 2001a). The United States Environmental Protection Agency for example, has determined that PAT, and the genetic material necessary for its production is exempt from the requirement to establish a maximum permissible level for residues in plants (EPA 1997).

2.1.2.2 Barstar and Barnase

193. The Barnase ribonuclease (RNase) protein in MS canola lines is expressed in a tissue-specific manner to produce a cytotoxic effect and thereby resulting in antherless, male

sterile plants. This cytotoxic effect is a result of the degradation of RNA in the cells in which it is expressed.

194. The Barnase protein being an RNase shares sequence homology with other ribonucleases. RNases are ubiquitous in nature (eg RNase is found on human skin), and are therefore commonly encountered and ingested in foods from a variety of sources. However, no homology was found to any known protein toxins (Van den Bulcke 1997).
195. The Barstar protein counteracts the RNase activity by binding to the Barnase protein (Hartley 1989). The Barstar protein does not share sequence homology to any of the known protein toxins (Van den Bulcke 1997).
196. The expression of the Barnase and Barstar proteins is restricted to developing flower buds. Both the barnase and barstar genes are under the control of an anther-specific promoter and are consequently expressed only in a single cell layer in the tapetum of developing anthers. The tapetum layer makes up only a small fraction of the total biomass of the plant. The level of expression of the Barnase or Barstar proteins in flower buds is very low and could not be detected in any other plant tissues (see Appendix 1 for details) such as leaves and seeds. Therefore, the likelihood of any exposure of humans to these proteins is remote.

2.1.2.3 NPTII protein

197. NPTII is an enzyme with a molecular weight of ~24 kD that catalyses the transfer of a phosphate group from adenosine 5'-triphosphate (ATP) to a hydroxyl group of aminoglycoside antibiotics, including neomycin, kanamycin and gentamicin A and B, thereby inactivating the antibiotics (Davies 1986).
198. NPTII is a commonly used marker protein that allows the selection of transformed plant cells early in the regeneration phase and can also be used in monitoring gene expression and genetic stability during later development of the plants.
199. In the lines that contain nptII gene, the protein is expressed at very low levels in leaves and none was detected in seed (see Appendix 1 for details). Since Bayer does not intend to commercialise any of these lines in Australia, the likelihood of exposure to the NPTII protein is negligible.
200. As previously mentioned, the NPTII protein is ubiquitous in the environment, in food chains, in naturally occurring kanamycin-resistant microorganisms found in soil and in mammalian digestive systems (Flavell et al. 1992).
201. The toxicity of the NPTII protein has been assessed by acute oral toxicity studies in mice. Fuchs et al (1993c) administered a single dose of purified NPTII protein to 10 male and 10 female mice to a maximum of 5000 mg/kg body weight (2500 mg/kg administered twice, four hours apart), followed by a seven day observation period. No mortality or adverse effects were observed. There were no treatment-related differences between treated and untreated mice in weight gain, food consumption, behaviour, clinical signs or gross pathology.
202. The potential toxicity of NPTII has been evaluated by a number of other regulatory authorities, both overseas and in Australia. Both INGARD[®] and Bollgard[®] II cotton express the NPTII protein and both have been approved for commercial release in Australia (IOGTR 2000c; OGTR 2002h). FSANZ has assessed a number of different GM food crops expressing the NPTII protein as safe (Davies 1986; ANZFA 2001b).

203. The Food and Drug Administration of USA (FDA 1994) concluded that “the use of aminoglycoside 3'-phosphotransferase II is safe for use as a processing aid in the development of new varieties of tomato, oilseed rape, and cotton intended for food use”. The Environmental Protection Agency determined that there is no requirement for a regulatory tolerance for NPTII expressed in plants (EPA 1994).
204. The safety of this protein has also been considered on numerous occasions in the peer reviewed scientific literature (Davies 1986; Flavell et al. 1992; Fuchs et al. 1993a; Fuchs et al. 1993b; Fuchs et al. 1993c).

2.1.3 Feeding studies

205. Feeding studies provide additional information on whether the toxicity of a GMO is altered as a result of genetic modification. They can also address the question of potential dietary toxicity and whether there are any unintended or 'pleiotropic' effects. A number of feeding studies was undertaken with the various GM canola lines.
206. One feeding study compared the performance of young broiler chickens fed with the canola seed from GM canola line Topas19/2 with those fed seed from a standard commercially available canola cultivar (Leeson 1999). Despite the presence of glucosinolates, whole canola seeds can be utilised as a major component in the diet of broiler chickens (Leeson 1999). Broiler chickens represent a very sensitive test species for dietary feeding studies because a 15 fold increase in body weight occurs during the first 18 days of life. Therefore, any differences in nutrient availability are readily detectable in terms of the development of the chickens (Leeson 1999).
207. The study involved the use of 280 commercial strain male broiler chickens obtained at one day of age fed a diet containing either Topas 19/2 canola seed or conventional canola seed. The variables considered were initial body weight, 18, 32 and 42-day body weight, body weight gain, feed intake, mortality rate and carcass characteristics at post-mortem. There were no differences between the chickens fed Topas 19/2 canola seed and those fed conventional canola seed for any of the measured parameters.
208. Two feeding studies were also conducted in rabbits to investigate the nutritive value of canola seed of hybrids derived from crosses of RF1 x MS1 (Maertens & Van Eeckhoutte 1994) and RF3 x MS8 (Maertens et al. 1996). Hybrids of RF1 x MS1 contain all four of the introduced genes – bar, barnase, barstar and nptII, while those of RF3 x MS8 do not contain the nptII gene.
209. Both studies compared the performance of rabbits fed a diet comprising 30% of either GM canola seed (RF1 x MS1 or RF3 x MS8) or seed from the non-GM, parental cultivar Drakkar. The study involved 10 rabbits per experimental diet. The rabbits were fed ad libitum (i.e. the animals were offered food and are able to feed at will). After a preliminary adaptation period of one week, faecal output was measured and recorded daily for the duration of the 4-day study.
210. No significant differences in feed intake, weight gain, final weight or feed efficiency were observed between either the GM canola diet or the Drakkar control diet. The individual faecal samples were analysed for dry matter, ash, nitrogen, fat and crude fibre following AOAC methods (Association of Official Analytical Chemists 1990).
211. Digestibility coefficients of protein, fat, crude fibre and bioavailable gross energy for the GM and non-GM canola seed were determined from the dry matter intake, output

and nutrient content. No significant differences were apparent in any of the parameters examined.

212. A feeding study conducted using canaries fed either GM canola seed from RF1xMS1 hybrids or conventional canola found no differences in food consumption, behaviour and body weight between the GM and non-GM diets (Canadian Food Inspection Agency 1995b).

2.1.4 Compositional analyses

213. Compositional analyses can provide evidence of whether any unintended effects have been introduced into the GM canola lines as a result of the genetic modifications; whether there are any significant changes with respect to processing characteristics, oil content, oil composition, oil quality (physical properties), or protein content.
214. Bayer have provided data from compositional analyses for all seven of the GM canola lines grown at various locations (De Both 1991a; De Both 1991b; De Both 1991c; De Both 1991d; De Both 1991e; De Both 1993a; De Both 1993b; De Both 1993c; MacDonald 1997; MacDonald 1998; Beriault 1999), including the results of experiments on RF3 and MS8 conducted in Australia (data supplied by Bayer). Because of the large amount of information provided, only representative data from GM canola lines RF3, MS8 and RF3xMS8 hybrids has been presented. It is only these lines that Bayer proposes to commercialise in Australia.
215. The levels of erucic acid and glucosinolates in all seven GM canola lines are below the industry standards and do not vary significantly from their parental cultivars or other commercially available canola.
216. Application of the herbicide glufosinate ammonium did not result in any significant differences in the levels of erucic acid or glucosinolates. Data (supplied by Bayer) on the erucic acid and glucosinolate content of seed from GM canola lines RF3, MS8 and RF3 x MS8 hybrids are presented in Tables 2 and 3 respectively.
217. Although some differences were observed, the variation across environmental conditions was greater than any variation between GM and non-GM canola plants. For example, in one of the experiments conducted in the UK, the glucosinolate levels were higher in all the lines including the non-transgenic control lines. This correlated with drought stress as the control lines showed similar increases suggesting that environmental factors have a major impact on the seed quality characteristics compared to the genotype. (De Both 1995a).
218. Compositional analyses demonstrate that the seven GM canola lines are comparable in composition (including fatty acid content, protein content and proximate analyses) to their parental cultivars, and to other commercial canola cultivars when grown at a variety of different locations, including Canada, Europe, UK and Australia (De Both 1991a; De Both 1991b; De Both 1991c; De Both 1991d; De Both 1991e; De Both 1993a; De Both 1993b; De Both 1993c; MacDonald 1997; MacDonald 1998; Beriault 1999). Application of the herbicide glufosinate ammonium did not have a significant effect on any of the compositional parameters investigated (De Both 1995b). Data on the fatty acid content and seed composition of GM canola lines RF3, MS8 and RF3 x MS8 hybrids are presented in Tables 2 and 3 respectively.

Table 2 Minimum and maximum values of fatty acid (% of total) in canola grown in North America and Europe in 1995 *(CODEX 2001)

Fatty Acid		CODEX*	Commercial	Parental cultivar	MS8	RF3	MS8xRF3
C16:0	Palmitic	2.5-7.0	4.1-5.3	3.9-5.2	3.9-4.8	3.9-5.1	3.9-4.5
C16:1	Palmitoleic	ND-0.6	0.3-0.4	0.0-0.4	0.3-0.4	0.3-0.4	0.2-0.3
C18:0	Stearic	0.8-3.0	1.5-1.9	1.6-2.1	1.5-1.8	1.5-1.7	1.6-1.8
C18:1	Oleic	51.0-70.0	57.7-66	60.8-68.4	60.1-67.6	58.2-67.4	60.9-67.4
C18:2	Linoleic	15.0-30.0	17.7-21.9	16.3-19.9	16.4-20.4	17.4-21.8	17.4-19.7
C18:3	Linolenic	5.0-14.0	8.1-12.1	6.2-10.7	7.3-10.9	6.6-11.6	7.0-11.1
C20:0	Arachidic	0.2-1.2	0.5-0.7	0.5-0.7	0.4-0.7	0.5-0.6	0.5-0.6
C20:1	Eicosenoic	0.1-4.3	1.0-1.6	0.9-1.4	0.9-1.5	1.0-1.6	1.0-1.5
C20:2	Eicosadienoic	ND-0.1	0.0-0.1	0.0-0.0	0.0-0.9	0.0-0.9	0.0-0.0
C22:0	Behenic	ND-0.6	0.0-0.4	0.0-0.4	0.2-0.4	0.0-0.4	0.0-0.4
C22:1	Erucic	ND-2.0	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0

Table 3 Seed Quality Analyses (Belgium 1995)

Canola Line	Oil (%)	Protein (%)		Glucosinolates (mmol alkenyls + indols/g)	
		seed	meal	seed	meal
MS8	37.99	30.54	51.18	27.29	45.74
RF3	37.33	30.90	51.30	27.80	46.15
MS8 x RF3	39.42	29.56	49.84	26.89	45.33
Control	38.47	30.12	50.45	26.75	44.81
Control	38.15	30.45	50.92	27.05	45.22

219. Data on the composition of RF3 x MS8 hybrids, which Bayer proposes to commercialise in Australia, have also been obtained under Australian conditions. Representative data are presented in Table 4. No significant differences were observed between RF3 x MS8 hybrids and non-GM commercial controls.

220. Glufosinate ammonium was applied to RF3 x MS8 hybrid canola lines at the Dooen field trial site (Victoria). On average, both the GM and non-GM lines in Dooen had lower oil content and higher protein content than the lines being trialled at Clear Lake (Victoria), due to different environmental conditions. The application of glufosinate ammonium did not result in an increase in either glucosinolates or erucic acid.

Table 4 Seed composition of RF3 x MS8 hybrids in Australia in 2000, 2001

	Ttl Glucosinolates whole seed 0% mb	Oil composition (% of total)				% whole seed	
		C18:1 Oleic	C18:2 Linoleic	C18:3 Linolenic	C22:1 Erucic	Protein	Oil
2000 Season							
Control: Cv Dunkeld Clear Lake, Victoria	9.17	59.96	19.77	10.91	0.03	21.03	47.06
RF3xMS8 Clear Lake, Victoria	9.52	60.78	19.71	10.23	0.32	19.17	49.37
Control: Cv Dunkeld Dooen, Victoria	10.89	58.58	20.07	11.87	0.04	24.00	43.87
RF3xMS8 + herbicide Dooen, Victoria	8.42	60.63	19.89	10.71	0.00	24.08	45.34

2001 Season							
Control: Cv Dunkeld Wagga, NSW	10.89	60.23	19.30	10.91	0.00	ND	ND
RF3xMS8 Wagga, NSW	8.96	61.20	19.33	10.14	0.00	ND	ND

ND = not determined

2.1.5 Toxicity of herbicide metabolites

221. The herbicide glufosinate ammonium comprises a racemic mixture of its L and D isomers. The L-isomer is the active constituent and acts by inhibiting glutamine synthetase. D-glufosinate ammonium does not exhibit herbicidal activity and is not metabolized by plants (Ruhland et al. 2002).
222. PAT is encoded by either the bar or the pat gene, inactivates the L-isomer of glufosinate ammonium by acetylating it to N-acetyl-L-glufosinate ammonium (NAG) which does not inhibit glutamine synthetase (Droge-Laser et al. 1994; Organisation for Economic Co-operation and Development 2002).
223. The metabolism of glufosinate ammonium in tolerant, genetically modified plants and in non-modified plants has recently been reviewed (Food and Agriculture Organization 1998; Organisation for Economic Co-operation and Development 2002). While in non-GM plants the metabolism of glufosinate ammonium is low to non-existent (because of plant death due to the herbicidal activity), some metabolism does occur (Muller et al. 2001) and is different to that in plants expressing PAT (Droge et al. 1992).
224. Two pathways for the metabolism of glufosinate ammonium in non-tolerant plants have been identified. The first step, common to both, is the rapid deamination of L-phosphinothricin to the unstable intermediate 4-methylphosphonico-2-oxo-butanoic acid (PPO). PPO is then either metabolized to:
- 3-methyl phosphinico-propionic acid (MPP, sometimes referred to as 3-hydroxy methyl phosphinoyl propionic acid) which may be further converted to 2-methyl phosphinico-acetic acid (MPA); or
 - 4-methylphosphonico-2-hydroxy-butanoic acid (MHB), which may be further converted to 4-methylphosphonico-butanoic acid (MPB), a final and stable product (Droge-Laser et al. 1994; Ruhland et al. 2002).
225. The main metabolite in non-GM plants is MPP (Muller et al. 2001; Organisation for Economic Co-operation and Development 2002).
226. The metabolism of glufosinate ammonium in genetically modified herbicide-tolerant canola has been investigated by Stumpf et al (1995) and Thalacker (1994, cited in Food and Agriculture Organization 1998). Both studies found that the major residue present in canola after glufosinate ammonium herbicide application was N-acetyl-L-glufosinate ammonium, with lower concentrations of glufosinate ammonium and MPP. By contrast, the residues found in unmodified canola were composed predominantly of the parent compound (80 %) with a small portion of MPP (Stumpf et al. 1995). This is consistent with data obtained from other plants expressing the PAT protein (Organisation for Economic Co-operation and Development 2002).
227. Studies using cell cultures of tolerant (GM) and sensitive canola yielded similar results, with N-acetyl-L-glufosinate ammonium the major metabolite in the glufosinate ammonium tolerant cells (Ruhland et al. 2002). D-glufosinate ammonium and

N-acetyl-L-glufosinate ammonium are readily transported in the phloem of glufosinate ammonium tolerant canola (Beriault et al. 1999).

228. N-acetyl-L-glufosinate and MPP are non-toxic to both plants and mammals, including humans (Organisation for Economic Co-operation and Development (OECD) 1999a; data supplied by Bayer, Organisation for Economic Co-operation and Development 2002). The toxicity of these metabolites was comparable to or less than that of the parent compound, and all were considered of low acute toxicity.

Section 2.2 Allergenicity

229. Allergens usually share a number of characteristics, including the following (Davies 1986; Flavell et al. 1992; Fuchs et al. 1993a; Fuchs et al. 1993b; Fuchs et al. 1993c; Taylor 1995; Fuchs & Astwood 1996; Metcalfe et al. 1996; Kimber et al. 1999; ANZFA 2001a):
- proteins;
 - molecular weight ranges between 15-70 kD;
 - typically glycosylated;
 - stable in the mammalian digestive system;
 - stable during the high temperatures involved in cooking or processing; and
 - and present as the major protein component in the specific food.
230. In assessing the likelihood of adverse impacts due to allergenicity of the seven GM canola lines, a number of factors were considered, including:
- allergenicity of conventional canola;
 - allergenicity of the new proteins expressed (PAT, Barnase, Barstar, NPTII); and
 - likely levels and routes of exposure to GM canola and the introduced proteins, for example in food or feed, in non-food products containing canola oil or meal or through direct contact with the crop or contact with soil in which the crop is grown.

2.2.1 Allergenicity of conventional canola

231. No allergic reaction to fats (including canola oil) has been reported by humans. Allergic sensitisation to canola can occur via the lungs (through inhaling pollen) or through skin contact (e.g. during handling). Further details can be obtained from the OGTR document on Biology and Ecology of Canola (OGTR 2002a).

2.2.2 Allergenicity of the introduced proteins

2.2.2.1 The PAT protein

232. The PAT protein is not a known allergen and is not derived from a known source of allergens. Although its molecular weight of ~22 kD is within the range of molecular weights usually shown by allergens, it lacks glycosylation sites (Bremmer & Leist 1996) and many of the other characteristics which are common to plant food allergens (EPA 1997; Canadian Food Inspection Agency 1998; ANZFA 2001a).
233. A study in which PAT protein was subjected to simulated gastric conditions (low pH plus the proteolytic enzyme pepsin) reported that the protein was degraded within seconds (Wehrmann et al. 1996). Data supplied by Bayer also clearly demonstrated that the PAT protein was rapidly degraded in simulated gastric fluid and simulated intestinal fluid conditions (proteolytic enzyme pancreatin), and loses activity and is denatured by temperatures over 40°C. Other studies have shown that the PAT enzyme

was inactivated within one minute when subjected to typical mammalian stomach conditions and was inactivated during processing of canola seed (from GM *B. napus* expressing the PAT enzyme) into feed ingredients (European Scientific Committee on Plants 1998a).

234. The USEPA (1997) reported that experimental data indicated that the PAT protein is rapidly degraded in the gastric environment and is also readily denatured by heat or low pH. Inactivation of PAT protein in bovine paunch fluid, which has a neutral pH (7.1), was slower but occurred within 30 minutes. Other studies have determined that the PAT enzyme is heat labile and is completely inactivated by temperatures above 75°C.
235. In addition, a study by Bremmer & Leist (1996) investigated the allergenicity of purified PAT protein in a repeated high-dose study in rats. The study did not reveal any immunotoxic allergenic effects based on a number of screening parameters (details of the study are discussed in Section 2.1.2.1).
236. The PAT enzyme does not constitute a major component of any tissues of GM canola lines T45, Topas 19/2, RF1, RF2, RF3, MS1 or MS8. PAT is expressed at low levels in green tissues and very low levels in seeds, therefore the exposure to the novel protein will be very low.

2.2.2.2 Barnase and Barstar proteins

237. The Barnase and Barstar proteins are not known allergens. The barnase and barstar genes are derived from *Bacillus amyloliquefaciens* which is not a known source of allergens and is used extensively in the industrial production of enzymes in the food industry.
238. The Barnase and Barstar proteins have molecular weights of 12 kD and 10 kD respectively (Wehrmann et al. 1996; Bremmer & Leist 1996), which toward the lower molecular weight range (15 - 70 kD) of known allergens (Fuchs & Astwood 1996).
239. However, purified Barnase and Barstar proteins are both rapidly degraded in simulated gastric conditions (0.32% pepsin and acidic pH), with all protein degraded within five minutes (Van den Bulcke 1997).
240. As noted in Section 2.1.2.2, ribonucleases and their inhibitors are ubiquitous in nature and therefore exposure to them is common.
241. The level of Barnase and Barstar proteins expressed in the RF and MS canola lines is extremely low, and restricted to the tapetum cell layer of developing anthers (see Appendix 1 for details). Therefore the likelihood of exposure to these proteins for humans is extremely low.

2.2.2.3 NPTII protein

242. The NPTII protein is not a known allergen and is not derived from a known source of allergens. The NPTII protein has a molecular weight of ~29 kD which is within the range exhibited by known allergens, however it does not display characteristics common to known food allergen proteins (Fuchs et al. 1993c; FDA 1998; ANZFA 1999).
243. The NPTII protein is heat labile (USDA-APHIS 2002d) and is very rapidly degraded by simulated gastric fluid (complete loss of detectable protein in <10 seconds) and simulated intestinal fluid (>50% loss of intact detectable protein after 5 minutes and complete loss of enzyme activity after 15 minutes, Fuchs et al. 1993c).

244. The rapid digestion of the NPTII protein means that it would not interfere with orally administered kanamycin or neomycin therapy because under normal gastric and intestinal conditions, it would be effectively degraded before the enzyme could inactivate kanamycin or neomycin (US FDA 1998; Fuchs et al. 1993c). In addition the enzymatic activity of NPTII requires the co-factor ATP which is unstable at the low pH of the digestive system (Flavell et al. 1992; Fuchs et al. 1993c). In the absence of ATP, NPTII cannot confer resistance to the aminoglycoside antibiotics.
245. The *nptII* gene is only present in GM canola lines Topas 19/2, RF1, RF2 and MS1. The NPTII protein is expressed at very low levels in leaves and is not detected in seeds. The *nptII* gene is not present in lines RF3 or MS8, the lines that Bayer proposes to release commercially in Australia.
246. As noted above, the safety aspects of the expression of the NPTII protein in plants have been assessed by a number of regulatory agencies, including the OGTR, FSANZ, United States FDA and EPA, and it has been concluded that NPTII enzyme does not have any of the recognised characteristics of food allergens or any attributes that would distinguish it toxicologically from other phosphorylating enzymes in the food supply (EPA 1994; Flavell et al. 1992; Fuchs et al. 1993c; FDA 1994; ANZFA 1999; OGTR 2002h).

2.2.3 Homology with known allergens

247. The amino acid sequences of the proteins encoded by the introduced genes; PAT, Barnase, Barstar and NPTII, were compared for overall homology with amino acid sequences of known allergens (aeroallergens and food allergens) of both plant and animal origin using various sequence databases. No significant homology to any known allergen was detected (Van den Bulcke 1997). Previous searches also revealed no homology of the NPTII protein with known allergens (Fuchs et al. 1993c).
248. Identified epitopes of allergenic proteins tend to have an optimal length of between 8 and 12 amino acids for binding to T-cells and it has been proposed that an immunologically significant sequence identity requires a match of at least eight contiguous amino acids (Metcalf et al. 1996). A search for homology with known allergens of the PAT, Barnase, Barstar and NPTII proteins was therefore conducted, based on detecting identities of eight contiguous amino acids and no sequence homologies were detected (Van den Bulcke 1997).
249. A more refined method for detecting possible allergenic epitopes has recently been published (Kleter & Peijnenburg 2002). The method is based on detecting identities of six amino acids with known IgE epitopes. The method was applied to the amino acid sequences of proteins introduced into GM plants, including the proteins in the Bayer canola lines: PAT, NPTII, Barnase and Barstar. No identities with known IgE epitopes were found confirming the previous results.
250. In addition, humans are extremely unlikely to be exposed to the proteins through the consumption of canola oil because of the stringency of the commercial processing in removing plant proteins from the final food product.

2.2.4 Exposure to pollen via honey

251. The possible exposure of humans to honey containing pollen from the GM canola and any implications for allergenicity was also considered.

252. Honey bees are a major pollinator of canola, and hives may be deployed in breeding, seed increase and general canola production (Gibbs & Muirhead 1998; Manning & Boldand 2000; OGTR 2002a). Flowering canola is primarily regarded as a breeding source for commercial apiaries in Australia, which is different from North America where it is regarded as a major nectar source.
253. The three factors against this plant being a major source of honey in Australia are cool weather in early spring, weaker populated colonies not capable of storing any large honey crops and use of pesticides on the crop is sufficient to deter many beekeepers moving bees onto this crop. Its main value to Australian beekeepers is as a source of pollen and stimulating nectar to induce the colony to expand. All the stored pollen would be consumed within a few weeks of the blossom finishing and in some years some honey would be extracted, although this honey is not regarded as table quality in Australia (Doug Somerville, personal communication)
254. Estimates of pollen content in commercial honey are well below 1%, typically in the range 0.006% to 0.3% (Malone 2002). The amount of pollen present also depends on whether the honey has been sieved, with sieving or filtering reducing the pollen content (Agrifood Awareness Australia 2001), sometimes to levels as low as 0.1% w/w (Malone 2002 and references cited therein).
255. Very low levels of protein from GM canola pollen have been detected in honey. A study by the UK Ministry of Agriculture, Fisheries and Food (MAFF) detected very low levels of novel protein in pollen in honey derived from GM canola. The study analysed honey samples (9 g) derived from a GM canola variety containing the nptII gene under the control of the nos promoter (MAFF 1997). The report did not specify whether the honey was sieved or filtered prior to analysis. NPTII protein was detected in pollen isolated from the honey by ELISA at a level of 1.61 ng/mg protein. Based on this result, the study calculated that a 500 g pot of the analysed canola honey would contain 0.00125 µg of NPTII protein (i.e. 0.0000025 ppm).
256. These results indicate that even if a transgenic protein were expressed in pollen, the level of exposure to such proteins that might occur from pollen presence in honey is extremely low. It should be noted that the GM canola line used in the MAFF study was not any of the seven GM canola lines being considered in this application. The introduced proteins in the GM canolas considered in this application are expressed at low to very low levels even in the plant tissues where expression is intended and no expression of the introduced genes has been detected in pollen (see Appendix 1 for details).
257. Most importantly, however, none of the introduced proteins PAT, Barnase, Barstar or NPTII in the seven GM canola lines are considered to be toxic or allergenic and these proteins will be commonly encountered in the environment. Therefore, the presence in honey of pollen from the GM canola lines would not represent any allergenicity risk to human health or safety.

2.2.5 Occupational exposure

258. Agricultural workers will be exposed to canola pollen. As noted in Section 2, (non-GM) canola pollen per se is implicated as a source of allergic reactions. However, the preceding sections have demonstrated that none of the introduced proteins is likely to be an allergen. No mRNA from any of the nptII, bar, barnase or barstar genes was detected in pollen (see Appendix 1 for details, FDA 1996a; European Scientific Committee on Plants 1998b; Health Canada 1999a; ANZFA 2001a). The introduced

proteins are expressed at low to very low levels even in the plant tissues where expression is intended (see Appendix 1 for details).

259. Occupational exposure to canola pollen (Chardin et al. 2001; OGTR 2002a), canola dust (Suh et al. 1998) and canola flour (Monsalve et al. 1997; Alvarez et al. 2001) have been implicated in allergic reactions in humans 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 conventional, non-transgenic canola, and that canola seed, meal or flour is not considered suitable for human food. The proposed commercial release does not include the use of GM canola seed, meal or flour for human food.
260. Bayer have stated in their application that their employees and contractors, who have been in daily contact with the GM canola plants, including the possible inhalation of significant amounts of GM canola pollen, have not shown changed allergic reactions (as compared to the non-GM canola) in annual medical examinations.

Section 2.3 Conclusions regarding toxicity and allergenicity

261. It is considered that the risk of any of the GM canola lines T45, Topas 19/2, RF1, RF2, RF3, MS1 or MS8 being toxic or allergenic to humans is very low because:
- Acute oral toxicity studies demonstrate that the PAT and NPTII proteins are not toxic, even at high doses;
 - PAT, Barnase, Barstar and NPTII proteins are all rapidly degraded by mammalian digestive systems;
 - The novel proteins do not share significant sequence homology with known protein toxins or allergens
 - Feeding studies demonstrate that there are no anti-nutritional effects of the genetic modifications in the GM canola;
 - The composition of the seven GM canola lines does not differ significantly from non-GM canola
 - The levels of the naturally occurring toxicants of canola, erucic acid and glucosinolates, do not vary between GM and non-GM canola;
 - The major metabolites of glufosinate ammonium are not toxic; and
 - All of the novel proteins, PAT, Barnase, Barstar and NPTII are expressed at low or very low levels. The Barnase and Barstar proteins are only expressed in the tapetum layer of developing flowers. The NPTII protein is not expressed in the lines RF3 or MS8 that are intended for commercialisation in Australia. Only the PAT protein is expressed in canola seed;
 - No expression of any of the introduced genes was detected in pollen;
 - Humans are commonly exposed to all of the novel proteins because they are derived from common bacteria and are naturally ubiquitous in the environment;
 - FSANZ has approved the use of oil derived from all seven GM canola lines in human food. Canola seed or meal is not used in human food;

APPENDIX 3 ENVIRONMENTAL SAFETY- TOXICITY TO OTHER ORGANISMS

262. Under section 51 of the Gene Technology Act 2000, the Regulator is required to consider risks to human health and safety or the environment in preparing the risk assessment and risk management plan. This part of the document considers potential toxicity hazards that may be posed to organisms other than humans. In this context, the potential toxicity of the GMOs and their novel proteins was considered.

SECTION 1 NATURE OF THE POTENTIAL TOXICITY OR ALLERGENICITY HAZARD

263. Potentially there could be impacts relating to the toxicity of the seven GM canola lines T45, Topas 19/2, RF1, RF2, RF3, MS1 and MS8 for:

- grazing animals, including native animals;
- animal feed safety, for example, animals fed canola seed or canola meal; and
- invertebrates (including insects) or soil biota, with direct impact on growth of crops on farms, as well as secondary ecological effects with potential to harm the natural environment (for example, adverse impacts on native biodiversity).

SECTION 2 LIKELIHOOD OF THE TOXICITY OR ALLERGENICITY HAZARD OCCURRING

264. In assessing the likelihood of adverse impacts due to toxicity of the seven GM canola lines for other organisms, a number of factors were considered, including:

- the toxicity of conventional canola;
- the toxicity of the new proteins (PAT, Barnase, Barstar, NPTII), and the potential for unintended changes in the levels of toxicants or nutritional factors;
- potential for altered metabolism of the herbicide; and
- information about the likely levels and routes of exposure to GM canola and the introduced proteins, for example in animal feed, or through direct contact with the crop or contact with soil in which the crop is grown.

265. When considering these factors, it is important to review the relevant conclusions of Appendix 2, which found that the risk of any of the GM canola lines being toxic to humans is very low because:

- Exposure to all of the novel proteins already occurs in nature as they are derived from common bacteria and are naturally ubiquitous in the environment;
- All of the novel proteins (PAT, Barnase, Barstar and NPTII) are expressed at low or very low levels in the tissues where they are expressed and none are expressed in pollen. The Barnase and Barstar proteins are only expressed in the tapetum layer of developing flowers. The NPTII protein is not expressed in the lines RF3 or MS8 intended for commercialisation in Australia. Only the PAT protein, responsible for glufosinate ammonium-tolerance, is expressed in canola seed;
- Acute oral toxicity studies in mice demonstrate that the PAT and NPTII proteins are not toxic, even at high doses;
- PAT, Barnase, Barstar and NPTII proteins are all rapidly degraded by mammalian digestive systems;

- The novel proteins do not share significant sequence homology with known protein toxins;
- Oil derived from all seven GM canola lines has been approved by FSANZ for use in human food;
- Feeding studies with whole canola seed using young broiler chickens, rabbits and canaries demonstrate that there are no anti-nutritional effects of the genetic modifications in the GM canola;
- The levels of the naturally occurring toxicants of canola, erucic acid and glucosinolates, do not vary between the seven GM canola lines and non-GM canola;
- The composition of the seven GM canola lines does not differ from non-GM canola; and
- The major metabolites produced by the inactivation of glufosinate ammonium by PAT protein are not toxic.

Section 2.1 Toxicity hazard of the GM canola for mammals and wildlife, including birds and fish

Exposure to the GM canola

266. Both the level and pattern of expression of the introduced proteins in the GM canola are important factors when considering the potential exposure (see Appendix 1 for details). Each of the introduced proteins is expressed at low to very low levels, and only in some tissues.
267. Bayer propose to commercialise only GM canola lines RF3 and MS8, and hybrids derived from these lines. Only the PAT, Barnase and Barstar proteins will be expressed in these lines.
268. Both bar and the pat genes encode the PAT protein (see Appendix 1 for details). In lines RF1, RF2, RF3, MS1 and MS8 expression of the bar gene is directed by the PSsuAra promoter which is expressed predominantly in green tissues. In lines T45 and Topas 19/2 expression of the pat gene is directed by the CaMV 35S promoter, which is considered to be a constitutive promoter
269. The Barnase protein is only expressed in GM canola lines MS1 and MS8 or MSxRF hybrids. The Barnase protein is only expressed in the tapetum layer of developing flower buds because the barnase gene is under the control of the tapetum-specific pTA29 promoter.
270. The Barstar protein is only expressed in GM canola lines RF1, RF2 and RF3 or MSxRF hybrids. The Barstar protein is only expressed in the tapetum layer of developing flower buds because the barstar gene is under the control of the tapetum-specific pTA29 promoter. GM canola lines T45 and Topas 19/2 do not express either the Barnase or Barstar proteins.
271. The NPTII protein is only expressed in GM canola lines Topas 19/2, RF1, RF2 and MS1. Bayer does not propose commercialisation of these lines. The nptII gene is under the control of the P-nos promoter from *A. tumefaciens*, which is considered as a weak constitutive promoter.
272. The expression of the introduced proteins in the seven GM canola lines is summarised in Table 1.

Table 1 Summary of expression of the introduced proteins in GM canola

	Leaves	Seed	Other tissues
PAT All lines	Low levels	Very low levels	Very low levels
BARNASE MS1, MS8 or MSxRF plants only	Not expressed	Not expressed	Flower buds only: tapetum layer of developing anthers
BARSTAR RF1, RF2, RF3 or MSxRF plants only	Not expressed	Not expressed	Flower buds only: tapetum layer of developing anthers
NPTII Topas 19/2, RF1, RF2, MS1 only	Very low levels	Not detected	Not detected

NB: None of the proteins are expressed in canola pollen

273. A range of animals may be exposed to the GM canola, including grazing animals. Birds, such as cockatoos and sparrows can shred or remove pods during development and maturity (Stanley & Marcroft 1999). However, birds do not feed on canola nectar. Mice can climb plants and feed on the seeds and pods, or feed on ungerminated seed sown close to the surface (Stanley & Marcroft 1999). Seed eating birds and mammals, such as mice, would be exposed to introduced proteins expressed in the seed. Native (eg kangaroos) or feral (eg hares or rabbits) grazing animals are likely to browse on canola plants. It is also possible that livestock may also intentionally or unintentionally be grazed on canola crops. Canola seed or pollen is not expected to enter aquatic habitats in any significant quantity, and therefore any exposure of aquatic species may be considered very low.
274. As discussed above, all of the introduced proteins (PAT, Barstar, Barnase, and NPTII) are derived from bacteria commonly found in natural environment such as the soil and water. Therefore, the proteins expressed by the GM canola are expected to be already widely present in nature and their presence in the GM canola is not expected to present any new toxicity risks to organisms in these environments. Ribonucleases (such as the Barnase protein) and ribonuclease inhibitors (such as the Barstar protein) are common in bacteria and plants and the same or similar proteins are normal parts of the diets of animals, humans and insects (USDA-APHIS 1999b).
275. The PAT protein is present in very low amounts in dry seed (eg 0.7 µg/g seed or 0.7 ppm) and the Barnase, Barstar and NPTII proteins are not detected in dry seed. In addition, the aim of canola production is to harvest as much of the seed as possible, although some canola seed is inevitably lost to the ground.
276. Barnase and Barstar are not expressed in leaves or other tissues. PAT and NPTII are present in low and very low levels respectively in leaves. PAT is detected at only very low levels in other tissues while NPTII is not expressed. As previously discussed, NPTII is not expressed in the lines proposed for commercial production in Australia. The low amount of the introduced proteins in vegetative tissues of the plants minimises exposure in the case of consumption of the green parts of the plant.

277. In addition, there are no reasons to expect that the bar, barnase or barstar genes will be expressed in pollen. The tapetum cell layer has a different cell lineage to the microspores that give rise to pollen (Koltunow et al. 1990; De Block & De Bouwer 1993). No mRNA expression from the nptII, bar, barstar or barnase was detected in GM canola pollen (data supplied by Bayer, FDA 1996a; European Scientific Committee on Plants 1998b; Health Canada 1999a; ANZFA 2001a).

2.1.1 Toxicity of the GM canola

278. Data from acute oral toxicity studies of the PAT and NPTII proteins in mice, feeding studies (in young broiler chickens, rabbits and canaries), compositional analyses, studies demonstrating the lability of the PAT, NPTII, Barnase and Barstar proteins in simulated mammalian digestive systems, and sequence homology analyses support the conclusion that the introduced proteins are not toxic and that there are no anti-nutritional effects of the genetic modifications in the seven GM canola lines. In addition, the major metabolites of glufosinate ammonium are not toxic (refer to Appendix 2 for more details).

279. A number of regulatory agencies have assessed whether the GM canola lines have any increased toxicity as a result of the genetic modifications. For example, in its assessment of GM canola line T45, the US APHIS stated that other glufosinate ammonium tolerant GM canolas have not been shown to be harmful to beneficial organisms or threatened and endangered species (USDA-APHIS 1998b).

2.1.2 Safety of feed for livestock

280. 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 (Canola Council of Canada 2001). Canola meal is a significant component of livestock feed in Australia, and is a rich source of protein for livestock (Queensland Department of Primary Industries 2002). Its usage has been growing rapidly in recent years, with the increase in availability as a result of increases in canola production and processing (Brennan et al. 1999). Full fat canola seed may also be used directly as animal feed (Roth-Maier 1999; Bertin et al. 1999).

281. Glucosinolates and erucic acid are naturally occurring toxicants in canola seed (Price et al. 1993). The effects of glucosinolates include thyroid, liver and kidney problems. Metabolites of glucosinolates can cause goitre in farm animals and are implicated in goitrogenic effects (Raybould & Moyes 2001). Erucic acid is implicated in cardiopathogenic effects (Charlton et al. 1975). Industry standards require canola meal to be low in glucosinolates (total glucosinolates of 30 μ moles/g toasted oil free meal, Organisation for Economic Co-operation and Development (OECD) 2001). The maximum level for erucic acid in canola seed is 2% in the oil fraction (CODEX 2001).

282. Glucosinolates remain in the canola meal after oil extraction while erucic acid is removed with the oil fraction during processing of canola seed. It is important to determine if the level of these known toxicants is altered in any of the seven GM canola lines.

283. Compositional analyses presented in Appendix 2 demonstrate that there are no significant changes in the GM canola lines with respect to erucic acid in seed or glucosinolates in the seed or meal. The levels of erucic acid and glucosinolates in the GM canola lines are below standard levels and do not vary significantly from their parental cultivars or other commercially available canola.

284. Feeding studies in young broiler chickens, rabbits and canaries using whole canola seed also support the conclusion that the genetic modifications in the GM canola lines have not resulted in any toxicity or anti-nutritional effects, and that the GM canola lines are comparable to conventional canola.
285. Acute oral toxicity studies in mice support the conclusion that neither the PAT nor NPTII proteins are toxic, with LD50s of greater than 2500 mg/kg body weight and 5000 mg/kg body weight respectively (see Appendix 2 for details). Analyses of the amino acid sequences of the PAT, Barnase, Barstar and NPTII proteins has revealed no sequence homology with any known toxins or allergens.
286. The production of canola meal involves a number of processes, including seed flaking, cooking, mechanical crushing to remove oil, solvent extraction of oil, desolventizing and toasting of the meal. Toasted canola meal is the most common fraction used as animal feed. Toasting of canola meal deactivates the enzyme myrosinase which is responsible for the production of toxic aglucone metabolites from glucosinolates such as thiocyanates, isothiocyanates and nitriles (Bell 1984; Canola Council of Canada 2001). Around 85% of canola meal available in Australia is produced via solvent extraction, and the remainder is from cold-pressed seed which may contain inhibitory levels of glucosinolates and myrosinase (Queensland Department of Primary Industries 2002).
287. As detailed above, the PAT protein is the only novel protein present in canola seed. High temperatures are employed in seed cooking (>80°C) and meal toasting (>100°C) which will inactivate the heat-labile PAT protein. PAT protein was detected by ELISA in GM canola seed from a cross of lines T45 and Topas 19/2, but no PAT was detected in toasted meal (data supplied by Bayer, ANZFA 2001a). A similar analysis of another glufosinate ammonium tolerant GM canola line, HCN10, found that no PAT protein could be detected by ELISA in either desolventised or toasted meal (data supplied by Bayer).
288. Canola from each of the seven lines has been assessed and approved for use in animal feed by regulatory agencies in Canada (Canadian Food Inspection Agency 1995a; Canadian Food Inspection Agency 1995b; refer to Table 2, Canadian Food Inspection Agency 1996b; Canadian Food Inspection Agency 1996d; Canadian Food Inspection Agency 2003), USA (FDA 1995; FDA 1996a; FDA 1997; FDA 1998) and Japan (Ministry of Agriculture 2000). Glufosinate ammonium tolerant canola and InVigor[®] hybrid canola based on the RF and MS lines have been approved for use in animal feed since 1995 and there have been no reports of adverse effects to livestock fed these GM canola lines.

Table 2 *Regulatory approval of GM canola lines for use in animal feed*

Line	Canada	USA	Japan
T45	1995	1998	1997
Topas 19/2	1995	1995	1996
RF1, MS1	1995	1996	1996
RF2, MS1	1995	1996	1997
RF3, MS8	1996	1996	1998

289. The European Scientific Committee for Plants in their analysis of lines RF3 and MS8 (1998b) concluded that “The amounts of PAT present in seed-meal fed to animals would be too low to cause even theoretical concern”.

290. A number of other crop plants (including sugar beet, soybean, maize and rice) have been genetically modified for glufosinate ammonium-tolerance via the introduction of the bar or pat genes. Regulatory approval in at least one country for use as human food and/or animal feed has been obtained for those crops (Aumaitre 2002). The barnase gene has been introduced into maize to produce male sterility and these GM maize varieties (MS3 and MS6) have also been approved for human food and animal feed in Canada (Canadian Food Inspection Agency 1996a; MS3, Health Canada 1997b) and the USA (MS3 and MS6, FDA 1996b; FDA 2000).
291. Analyses of the composition of GM glufosinate ammonium tolerant maize and sugar beet, and their digestibility and nutritional value in sheep and pigs have concluded that the GM crops were not only substantially equivalent, but nutritionally equivalent to feed derived from non-GM sugar beet and maize (Flachowsky & Aulrich 2001; Bohme et al. 2001; Aumaitre et al. 2002).
292. Aumaitre et al (2002) undertook a comprehensive review of the nutritional equivalence and safety of GM feeds and concluded:
“Compositional analysis has always shown the genetically modified plants to fall within the range of established values. The equivalence in digestible energy and crude protein between isogenic and transformed plants expressing a wide range of modifications (insect resistance, herbicide tolerance, or the *barnase/barstar* system of sterility/fertility restoration genes) also has been clearly demonstrated in different species. In none of these experiments, whether measured as growth rate, feed efficiency and carcass merit in beef cattle, egg mass in laying hens, milk production, composition and quality in dairy cows or digestibility in rabbits, affected feeding transformed plants compared to animals fed control or isogenic plants.”
293. These results further support the conclusion that the genetic modifications introduced to the GM canola lines have not resulted in any increased toxicity or anti-nutritional effects and that they will be as safe as conventional canola when consumed by livestock or other animals.

Section 2.2 Toxicity hazard of the GM canola for invertebrates (including insects), microbes and soil biota

Soil invertebrates including insects

294. A number of studies have investigated the effect of GM glufosinate ammonium tolerant canola on epigeal fauna (invertebrates living or occurring on or near the surface of the ground) through comparisons with conventional canola. One study sampled nearly 29,000 specimens of spiders, carabids and staphylinid beetles from more than 300 different species in Germany between 1994 and 1996 (Volkmar et al. 1999). The investigation included species composition, population density and activity behaviour of these important epigeous predatory arthropods and found no differences between the GM canola PHY/22 (containing the bar gene and the hybrid breeding system) and non-GM canola. It concluded that the growing of the GM canola and the application of glufosinate ammonium herbicide had not led to any recordable changes in the ecological structure.
295. Another study was conducted with glufosinate ammonium tolerant canola in Germany between 1996 and 1998 to investigate the consequences for the epigeal fauna from the application of glufosinate ammonium (Volkmar et al. 2000). It included hybrid GM canola PGS-W1 (RF1xMS1) and conventional canola plants. A high diversity of

species and a considerable biological activity was observed in both years of the study. There were statistically significant differences of arthropod activity in the GM glufosinate ammonium tolerant canola, which were caused by the different levels of weeds resulting from herbicide usage. No evidence could be established for an impairment of epigeal fauna caused by the cultivation of GM glufosinate ammonium tolerant canola.

296. A further study in Germany captured 81,054 arthropod individuals from 208 species using pit-fall traps over a three year period (Volkmar et al. 2001). This study found no evidence for an impairment of the epigeal fauna in connection with the cultivation of GM glufosinate ammonium tolerant canola.
297. USDA-APHIS in one of its reports, concluded that knowledge of the mode of action, and the lack of known toxicity of the newly expressed proteins in the GM canola suggest no potential for deleterious effects on beneficial organisms such as earthworms (USDA-APHIS 1999b).
298. Bayer has stated in their application that there has been no observed change in the ability of the GM canola lines to add or subtract substances from the soil in trials conducted in Australia, including the 5 years of trials of the lines MS8 and RF3 proposed for commercialisation in Australia as InVigor[®] canola. Most past trial sites contain both GM and non-GM material which would allow obvious differences in the capacity to add and remove substances to be detected. Use of past trial sites range from the cropping cereals to grazing and fallow, without any obvious detrimental or advantageous effects being detected between GM and non-GM canola. Bayer has also stated that there has been no observed difference in the biodegradability of the GM and non-GM canola and no effects on native species present or near trial sites or abundance of prey or parasites at any of the trials sites conducted to date in Australia.

2.2.1 Soil microbes

299. Several studies have investigated the effect of growing GM glufosinate ammonium tolerant canola on soil microbes by a variety of means.
300. Gyamfi et al (2002) conducted experiments in a contained environment (pots) to investigate possible shifts in eubacterial and *Pseudomonas* community structures in the rhizosphere (the soil zone that surrounds and is influenced by the roots of plants) due to the presence of glufosinate ammonium tolerant canola variety Liberator C/6AC (which contains the pat gene). They assayed the community structures using denaturing gradient gel electrophoresis of 16S rRNA gene fragments amplified from rhizosphere DNA using eubacterial and *Pseudomonas*-specific PCR primers. A range of different scenarios was studied, including GM glufosinate ammonium tolerant canola versus conventional canola with and without the application of glufosinate ammonium or metazachlor (a chloroacetamide) herbicides, as well as with mechanical removal of weeds.
301. The results revealed slightly altered microbial communities in the rhizosphere of the GM canola plants. Importantly however, these effects were minor when compared to the shifts that occurred in both GM and non-GM plants in a manner correlated with the developmental stage of the plants such as flowering and senescence. As the presence of the pat gene does not suggest a priori effects on the micro-flora, the authors suggest that the slight differences between the GM and conventional lines were most likely due to unintended effects in the GM plants on characteristics such as altered root exudation (Gyamfi et al. 2002).

302. Application of either glufosinate ammonium or metazachlor herbicides caused transient changes in the eubacterial and *Pseudomonas* population structure, possibly due to the enrichment of microbes involved in degrading the herbicides and inhibition of sensitive organisms. This observation is not unexpected as the herbicidal isomer L-glufosinate ammonium is produced naturally as an antibiotic by *Streptomyces* spp. such as *S. hygrosopicus* and *S. viridichromogenes* and which also possess the PAT enzyme for detoxification (Hoerlein 1994). Metazachlor is also be metabolised by soil microflora (Beulke & Malkomes 2001).
303. Becker et al (2001) studied the diversity of the nitrogen fixing bacterium *Rhizobium leguminosarum* bv. *viciae* in German fields cultivated with GM glufosinate ammonium tolerant canola, compared to conventional canola. In summer trials they found that although some strains were found in soils around the GM canola that were not detected with the conventional canola, and some GM canola lines showed a higher *Rhizobium* diversity, no significant effects on rhizobial numbers or basal respiration were observed. In winter trials they reported no significant differences in the *R. leguminosarum* between the GM and non-GM canola (Becker et al. 2001)
304. Dunfield and Germida (2001) compared the microbial composition of the rhizosphere and root interior of a number of different non-GM and GM canola varieties in Canada, including three glufosinate ammonium-tolerant varieties (InVigor, Innovator, Exceed), by fatty acid methyl ester analysis (FAME) and community level physiological profiles (CLPP). Analyses were done on plants at the flowering stage. They found that total colony forming units (CFU) of rhizosphere and root interior microbial communities did not vary between the GM and non-GM canola varieties. However some differences in FAME and CLPP for the rhizosphere and root interior communities were observed between the GM glufosinate ammonium tolerant and non-GM varieties, although the differences appeared to be significantly influenced by soil type.
305. The effect of GM canola lines RF1, RF2, MS1 and MS1xRF2 hybrids on bacteria in the rhizosphere was investigated at the flowering and seed maturity stages. No significant differences between GM and non-GM canola lines were observed for population density of rhizobacterial flora (as determined by CFU) or community composition (as determined by protein fingerprint types), but that population shifts occur with changes in plant development (Canadian Food Inspection Agency 1995b, data supplied by Bayer).
306. Schmalenberger and Tebbe have investigated the effect of the presence of the *pat* gene in GM glufosinate ammonium tolerant maize and sugar beet on rhizosphere microflora by using a genetic profiling technique based on PCR amplification of 16S ribosomal RNA genes and single-strand conformation polymorphism (PCR-SCCP). No significant differences were observed in microbial communities between GM and non-GM plants of maize or sugar beet, nor was there any significant effect of the application of glufosinate ammonium herbicide (Schmalenberger & Tebbe 2002; Schmalenberger & Tebbe 2003a; Schmalenberger & Tebbe 2003b). Differences in the microflora were observed for maize plants (both GM and non-GM) at different growth stages (Schmalenberger & Tebbe 2002) and for sugar beets between seasons and also between site heterogeneity (Schmalenberger & Tebbe 2003b).
307. *Streptomyces hygrosopicus*, the source of the bacterial bar gene, *Streptomyces viridichromogenes*, the source of *pat* gene and *Bacillus amyloliquefaciens*, the source of the barnase and barstar genes are common soil bacteria. Therefore, all the introduced proteins are expected to be already found in the soil. The Barnase protein is naturally

excreted into the extracellular environment by *Bacillus amyloliquefaciens*, while the Barstar protein is an intracellular protein (Hartley 1989). The source of the nptII gene, *Escherichia coli*, is also a commensal bacterium of the human gut and commonly encountered in the environment. NPTII protein is not expressed in the lines proposed for commercial production.

308. In addition, as discussed above, the introduced proteins are expressed at very low levels, as intracellular proteins within the double walled plant cells. The barstar and barnase genes are not expressed anywhere except the tapetal cell layer (during anther development) and so exposure of organisms in soil to residues of these proteins is unlikely to occur as a result of root exudations.

2.2.2 Insects

309. Canola may be grazed by a wide range of insects including cabbage moths (*Plutella xylostella*), heliothis caterpillars (Family Noctuidae) and aphids (eg. *Brevicoryne brassicae*, *Lipaphis erysimi*). The lack of known toxicity of the newly expressed proteins and their low expression suggest that feeding on the plants will not unduly affect the ability of these insects to reproduce or function normally after feeding (USDA-APHIS 1999b).
310. Honey bees are a major pollinator of canola and hives may be deployed in breeding, seed increase and general canola production (Gibbs & Muirhead 1998; Manning & Boldand 2000; OGTR 2002a). Flower nectaries provide a source of nutrients for pollinators and flowering canola represents a major beekeeping floral resource in Australia, mostly for pollen for bee nutrition, particularly in the early months of spring (Somerville 1999; Somerville 2001; HoneyBee Australis 2001; Goodman 2001; Somerville 2002).
311. Bayer has reported that the nectaries in the flowers of the seven GM canola lines develop normally and insect activity was also normal. GM canola lines MS1 and MS8 lack anthers and do not produce pollen. Pollen production in the other GM canola lines is no different to non-GM canola. Hybrid plants resulting from MSxRF crosses also have normal flower morphology, fertility, and attractiveness to insect pollinators and normal insect activity was observed on all these plants (USDA-APHIS 1999b).
312. In its assessment of the GM canola lines MS8 and RF3, USDA-APHIS concluded that there is no reason to believe that deleterious effects on beneficial organisms could result from the cultivation of MS8 or RF3 canola or their hybrids, and that the trait controlling male sterility affects only anther and pollen development; while flower nectaries develop normally, and the flowers do not show a greater tendency towards bud abortion (USDA-APHIS 1999b).
313. A study on the foraging behaviour of bees on either MS1xRF1 hybrid GM canola plants or conventional canola found no significant differences (Canadian Food Inspection Agency 1995b, data supplied by Bayer). Similarly, no negative effects on the foraging or brood behaviour of bees have been observed in the lines T45 (USDA-APHIS 1998b), Topas 19/2 (Canadian Food Inspection Agency 1995a; European Scientific Committee on Plants 1998a), or RF3 and MS8 (European Scientific Committee on Plants 1998b; USDA-APHIS 1999b).
314. Other studies have not found any negative impacts on bees foraging on glufosinate ammonium tolerant canola plants (Malone 2002). A study by Chaline et al (cited in, Malone & Pham-Delegue 2001) found no significant differences between GM

glufosinate ammonium tolerant canola plants expressing the pat gene and conventional canola with respect to flower number, nectar volume or sugar concentration, or in worker bee mortality, foraging activity or colony health. Field studies by Pham-Delegue et al (2002) comparing glufosinate ammonium tolerant canola (pat gene) and the equivalent non-GM varieties did not detect significant differences in the total number of insects (honey bees, bumble bees or dipterans) visiting flowers or any negative impacts. However they did observe increased foraging of honey bees on the GM canola, and a tendency for increased nectar secretion with higher sugar content in the particular GM lines tested in one season. The observed differences in nectar production were not evident in the following season (Pham-Delegue et al. 2002).

315. Assessments by other regulators and advisory bodies have all concluded that none of the seven GM canola lines are likely to impact on other organisms (Canadian Food Inspection Agency 1995a; Canadian Food Inspection Agency 1995b; Canadian Food Inspection Agency 1996b; Canadian Food Inspection Agency 1996c; Canadian Food Inspection Agency 1996d; European Scientific Committee on Plants 1998a; USDA-APHIS 1998a; European Scientific Committee on Plants 1998b; USDA-APHIS 1999a; USDA-APHIS 2002b; USDA-APHIS 2002c).
316. For example, USDA-APHIS in its assessment of GM canola lines MS8 and RF3 concluded that knowledge of the mode of action, and the lack of known toxicity for the newly expressed proteins suggest no potential for deleterious effects on beneficial organisms such as bees. The male sterile line, fertility restorer line and their hybrid cross do not contain elevated levels of toxic oils. Therefore, insects that may feed on these canola will not be unduly affected in their ability to reproduce or function normally after feeding. Results of trials in the United States, Canada, and Europe do not reveal any noticeable adverse effects on beneficial organisms. APHIS concluded that the cultivation of male sterile line, fertility restorer line canola will not have deleterious effects, either directly or indirectly on organisms that are recognised as beneficial to agriculture or on threatened and endangered species (USDA-APHIS 1999b).

Section 2.3 Conclusions regarding toxicity to other organisms

317. The following summary strongly supports the conclusion that the GM canola lines T45, Topas 19/2, RF1, RF2, RF3, MS1 and MS8 will not present a toxicity or allergenicity hazard to any organism such as:

agricultural or native animals (vertebrates)

- Proteins produced by the introduced genes, PAT, Barnase, Barstar and NPTII, occur naturally in soil and water organisms;
- expression in plant tissues (where present) is at low or very low levels;
- not toxins or allergens;
- do not share significant sequence homology with known protein toxins or allergens;
- are all rapidly degraded by mammalian digestive systems;
- the major metabolites of the glufosinate ammonium herbicide are not toxic;
- nutritional composition of the plants has not changed significantly;
- levels of the naturally occurring toxicants of canola, erucic acid and glucosinolates, do not vary between GM and non-GM canola;
- only the PAT protein is expressed in canola seed which is destroyed by the normal processing of canola seed to produce meal for use in animal feed;

- digestibility and nutritional value of the GM canola seed is not different to conventional canola;
- feeding studies in a range of animals, including rabbits, broiler chickens and canaries demonstrate that there are no toxic or anti-nutritional effects of the genetic modifications;
- feeding studies in pigs and sheep with other glufosinate ammonium tolerant GM crop plants also demonstrate that there are no anti-nutritional effects associated with the presence of the PAT protein; and
- there are no reports of adverse effects of the GM canola lines on native animals or birds during trials in Australia or commercial production in North America.

insects (especially honey bees)

- floral and nectary development is normal in all seven GM lines and MSxRF hybrids;
- pollen production is normal in GM canola lines T45, Topas 19/2, RF1, RF2 and RF3 and MSxRF hybrids; GM canola lines MS1 and MS8 lack anthers and do not produce pollen. MS lines are only grown alone for breeding and seed increase;
- no differences between the behaviour and health of bees foraging on the GM canola lines or non-GM canola; and
- studies with other GM glufosinate ammonium tolerant canola lines expressing the PAT protein have found no adverse impacts on foraging bees.

other invertebrates, microbes and soil biota

- no significant differences were found in the numbers of soil arthropods, including spiders and beetles, between RF1xMS1 hybrid GM canola and non-GM canola, nor did glufosinate ammonium application result in significant differences;
- no reports of adverse effects of the GM canola lines on invertebrates during trials in Australia or commercial production in North America;
- all of the introduced genes are derived from commonly occurring soil or commensal bacteria and the encoded proteins are already be present in soil;
- Barnase protein is naturally excreted by *Bacillus amyloliquefaciens* but its expression in the GM canola plants is restricted to specific cells in developing flowers;
- the proteins produced by the introduced genes are expressed at low to very low levels in the GM canola plants;
- no differences have been detected in soil microbe populations between the GM canola lines and non-GM canola or any significant differences as a result of glufosinate ammonium herbicide application; and
- no differences in soil microbes associated with the presence of the PAT protein, nor was there any observed effect of the application of glufosinate ammonium herbicide.

APPENDIX 4 ENVIRONMENTAL SAFETY - WEEDINESS

318. Under section 51 of the Gene Technology Act 2000, the Regulator is required to consider risks to human health and safety or the environment in preparing the risk assessment and the risk management plan. This part of the document considers potential hazards that may be posed to the environment. In this context, the potential weediness of the GMO was considered.
319. There are numerous definitions of weeds including 'a plant growing where it should not be'. Weeds become a problem to the community when their presence or abundance interferes with the intended use of the land they occupy. Weeds may also represent a source of food to various organisms hence the introduction of weeds to an environment may also bring about ecological change by altering the structure of food webs.
320. Typically weeds are plant species that spread easily in disturbed areas or among crops. Weeds generally have a range of life history characters in common that enable them to rapidly colonise and persist in an ecosystem. These characteristics include the following:
- germination and seed production under a wide range of environmental conditions;
 - long-lived seeds with extended dormancy periods;
 - rapid seedling growth;
 - rapid growth to reproductive stage;
 - long continuous seed production;
 - self-pollinating but not exclusively autogamous;
 - use of unspecialised pollinators or wind when outcrossing;
 - high seed output under favourable conditions;
 - special adaptations for long distance and short distance dispersal; and
 - good competitiveness (Baker 1965; Baker 1974).
321. It is generally accepted that most crop plants, including canola, have undergone selective breeding and domestication, resulting in reduced competitiveness. Crop plants tend to function optimally only under controlled agricultural conditions or in areas where regular disturbance occurs.

SECTION 1 NATURE OF THE WEEDINESS HAZARD

322. Bayer sought regulatory approval for seven (7) genetically modified lines of canola: T45, Topas 19/2, MS1, RF1, RF2, RF3 and MS8. All of the Bayer GM canola lines have been approved for food use in Australia (ANZFA 2001a). Lines MS1, MS8, RF1, RF2 and RF3 and hybrids derived from MS x RF crosses are covered by the registered trade name InVigor[®] canola.
323. All seven of the GM canola lines have been genetically modified to introduce tolerance to the herbicide glufosinate ammonium. Five of the GM canola lines, RF1, RF2, RF3, MS1 and MS8, have been modified to introduce a novel hybrid breeding system for canola, based on genetically modified male sterile (MS) and fertility restorer (RF) lines.
324. Four of the seven lines have also been modified to introduce an antibiotic resistance marker gene (nptII).
325. Bayer has indicated that it intends to commercialise only InVigor[®] hybrid canola derived from crosses of lines RF3 and MS8 in Australia, but sought approval for all

seven lines to achieve consistency with existing Australian and overseas regulatory approvals.

326. All seven GM canola lines were considered with respect to weediness, however given the fact that only lines RF3 and MS8 are intended for commercialisation in Australia, particular attention is given to these lines.
327. This risk assessment investigates the potential for the GM canola lines to be harmful to the environment due to possible weediness or increased potential for weediness.
328. This assessment also evaluates the possibility that the genetic modification has, either directly or as a result of 'pleiotropic' effects, increased the weediness of the canola plants. This could result from changes such as increased fitness due to higher levels of herbicide resistance or increased fecundity.
329. This assessment has adopted a precautionary approach and considered whether the commercial scale release of the GM canola without any specific containment or management conditions poses a risk of weediness impacts to the environment.

SECTION 2 LIKELIHOOD OF THE WEEDINESS HAZARD OCCURRING

Section 2.1 Inherent Weediness of Conventional canola

330. Canola has a number of life history traits in common with those usually associated with weeds. Canola:
 - is able to grow under a wide range of environmental conditions
 - has seeds that can be induced into secondary dormancy and survive in the soil for several years
 - is self-pollinating but is not exclusively autogamous
 - uses unspecialised pollinators or wind when outcrossing and
 - has high seed output under favourable conditions
331. Although canola has a number of 'weedy traits', it is a poor competitor and does not establish well in unmanaged areas (Salisbury 2002). Canola occurs in disturbed habitats along roadsides, railway lines, field margins and waste lands in all countries where it is grown. It is not considered invasive and its dissemination normally results from seed spillage during harvest and transport operations. It has been reported as a minor agricultural problem in southern Australia (Groves et al. 2000), Canada (Canadian Food Inspection Agency 1994) and the U.S.A (Weed Science Society of America 1992).
332. Weeds which occur on farms have different characteristics to those that occur in undisturbed natural habitats. An analysis of data sets worldwide indicated that agricultural weeds tend to be herbaceous, rapidly reproducing, abiotically dispersed species, while weeds of undisturbed natural environments were primarily aquatic or semi-aquatic, grasses, nitrogen-fixers, climbers, and clonal trees (Daehler 1998). Canola's behaviour in relation to both these habitats are discussed in detail below.

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333. Canola can produce large numbers of small seeds (average seed weight is 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. These seed losses often result in high densities of plants occurring as weeds ('volunteers') in subsequent crops (Legere et al. 2001). Harvest seed losses of 1.5 to 8.5 % of the average yield have been reported

- in France (CETIOM 2000) and 3.3 to 9.9% in Canada {Gulden, 2003 3384 /id}. With an average canola yield of 1.5t/ha in Australia and Canada and a slightly smaller seed size, this would equate to 675 - 3,825 seeds/m² (Salisbury 2002). Seed loss at harvest can be reduced by: windrowing at about 20 - 35 % seed colour change to decrease shatter loss; properly adjusting combines to minimise seed loss; and by lowering combine speed (Thomas 2000). Gulden et al. {Gulden, 2003 3384 /id /d} noted that improper harvester settings and excessive harvester speed can contribute to significant harvest losses, and they that improved harvest management can reduce additions to the canola seed bank. The majority of the Australian crop is windrowed to avoid seed losses through pod shatter at harvest (Walton et al. 1999).
334. At maturity, canola seed exhibits no sign of dormancy. However, if environmental conditions are unfavourable for germination secondary dormancy can be induced (Lutman 1993). Factors shown to induce secondary dormancy include exposure to darkness, temperatures above 20°C, and low soil water availability and sub-optimal oxygen in darkness (Pekrun et al. 1997b; Linder 1998; Lopez-Granados & Lutman 1998; Gulden et al. 2000; Momoh et al. 2002). Secondary dormancy can be broken by low temperatures (2 - 4°C)(Gulden et al. 2000) or by alternating warm and cold temperatures (Pekrun et al. 1997a; Squire 1999). The development of secondary dormancy can vary considerably between cultivars and even between seed lots from the same cultivar (Pekrun et al. 1997c; Lopez-Granados & Lutman 1998; Gulden et al. 2000; Momoh et al. 2002). Compared to wild relatives, the survival of canola seed in the seedbank is very low (Hails et al. 1997).
335. Soil type also influences secondary dormancy (Pekrun et al. 1998). In a study in the UK, seed was distributed on cultivated soil at 2 field sites with different soil types; flinty silty clay loam and sandy soil. After 8 months, the seedbanks in the sandy soil were much larger than in the clay loam. The main reason was presumably the difference in soil texture and associated difference in water availability, the sandy soil retaining less moisture. Laboratory studies showed that the proportion of dormant seeds tended to rise with decreasing water potential.
336. Light sensitivity can also develop in canola enabling the seed to germinate in response to very short exposure to light, as experienced during soil cultivation. It has been recommended to retain seed on the soil surface for as long as possible to avoid seed persistence in the soil (Lopez-Granados & Lutman 1998, C. Preston pers. comm.), however light sensitivity can also develop in low tillage situations where large quantities of crop residue create shaded conditions (Legere et al. 2001).
337. In the majority of canola growing regions of Australia, where high temperatures and low soil moisture occur after harvest, seed is exposed to unfavourable conditions for germination. These conditions may be more favourable for the development of secondary dormancy than in the Northern hemisphere where conditions after harvest are cool and moist (J. Baker personal communication). Some canola growing areas, such as Tasmania and parts of southern Victoria and South Australia, may experience post-harvest conditions similar to those in the Northern hemisphere.
338. Canola has the ability to persist in the seedbank for several years allowing the emergence of volunteers over a prolonged period. Canadian studies have shown that seed bank density in cultivated fields declined ten-fold in the first year after harvest, but only declined slowly thereafter with low densities (0.2 to 0.5 plants/m²) of volunteers present in fields 4-5 years after a canola crop (Legere et al. 2001; Simard et al. 2002). Seasonal variation in seed bank density in Canada occurred as a result of seeds being

produced by volunteer plants each spring thereby replenishing the seed (Legere et al. 2001).

UNCROPPED DISTURBED HABITATS

Northern Hemisphere

339. Canola is not considered an invasive weed and its dissemination normally results from seed spillage during harvest and transport operations.
340. Persistence of canola seed is considerably longer in undisturbed soils compared to cultivated soils (Chadoeuf et al. 1998), most likely due to tillage and activation of germination by exposure to light in disturbed soils. In France, a conventional oilseed rape cultivar that had not been commercially grown by farmers in the region for 8 years was recorded on road verges surrounding a grain silo (Pessel et al. 2001). The persistence of this variety was considered to be the result of late germination of dormant seeds since any recruitment of plants would most likely involve hybridisation with new cultivars that provide the overwhelming source of pollen. Old varieties of oilseed rape were detected in Scottish feral populations 5-10 years after they were last commercially cultivated indicating either self-sustaining populations or a persistent viable seedbank (Squire et al. 1999). Persistence over an extended time may also suggest that the presence of canola in these locations was not considered a problem and the canola was not subject to active management.
341. Feral canola plants can increase the seedbank in the area immediately surrounding the plants. In four of the six populations sampled in Angus and Fife in eastern Scotland, the seed content of soil cores taken after pod maturation and seed dispersal were greater than those taken beforehand indicating that feral canola populations are capable of being self-sustaining (Wilkinson et al. 1995).
342. Mapping of the location and size of feral populations in Scotland over 3 years found that there was a large turnover of populations between years (Wilkinson et al. 1995). Although none of the populations were present during all three years, five were present in 1993 and 1995 after being absent in 1994. The reappearance of such populations may be attributed to fresh seed spillage in the same location or to germination from a viable seedbank. Other UK population studies showed that the persistence of canola on roadsides by local recruitment, without disturbance, is about 3 or 4 years and that the density of feral populations on roadsides correlated with human activities, especially with the transport of seed by trucks (Crawley & Brown 1995).

Australia

343. A survey for the presence of canola plants was conducted in September/November 2001 along 4000km of representative roadsides in the major canola growing regions of Australia, including New South Wales, Victoria, Western Australia, South Australia and Tasmania (Agrisearch 2001). This survey was conducted once and therefore the data collected represents a snapshot of the distribution of canola in these areas. Observations were made every 10km in an area 20m by 5m (100m²). The results of the survey showed that canola was recorded at 31%, 20% and 13% of survey points in southern New South Wales, Western Australia and Victoria, respectively. It was reported in 9% of survey points in South Australia, 4% in Tasmania and was not observed at all in northern New South Wales. The density of the canola plants was low and the plants were small. It was observed that in the majority of cases canola was only

found within the first 5m from the roadside and not beyond, indicating that initial spread of seed was from transport along roads. The survey found no evidence of persistence of canola through self-sustaining populations (Agrisearch 2001).

344. Another survey was conducted throughout Australia, with emphasis on canola growing areas, by interviewing council, road and rail authorities, and National Park weed personnel (Dignam 2001). Most of the areas managed by respondents were not treated at all, being 59% of council lands, 56% for road and rail and 93% of National Parks. Canola was only reported by 8% of road and rail authorities when respondents were asked to list their main weed types. When prompted with a list of weeds, canola was reported by 30% of councils, 4% of road and rail authorities and was not reported as occurring in National Parks. Only 5% of councils and 4% of road and rail authorities reported canola being present in large numbers. Of those reporting canola, approximately 70% did nothing to control it while those seeking to control it mainly used glyphosate.

UNDISTURBED NATURAL HABITATS

345. There are no studies which provide evidence that canola is a significant or invasive weed of natural ecosystems in Canada (Canadian Food Inspection Agency 1994; Warwick & Small 1999; Beckie et al. 2001) or Australia (Salisbury 2002). Due to selective breeding and domestication, crop plants only function optimally under controlled agricultural conditions and, therefore, pose no threat to biodiversity in undisturbed habitats such as National Parks, State Forests or remnant vegetation areas (Crawley et al. 2001). In a UK study in 8 different undisturbed natural habitats over 10 years, canola was shown to decline in abundance after the first year and no populations persisted for more than 3 years (Crawley et al. 2001). As noted above, canola was not reported as occurring in National Parks in the major canola-growing areas of Australia (Dignam 2001).

Section 2.2 Weediness of the GM canola lines

346. There is no evidence that the new traits introduced into the seven GM canola lines (including the hybrid system) would cause any of these canola lines to be more weedy than conventional canola. The RF3 and MS8 hybrid canola lines proposed for commercialisation in Australia have been developed using elite Australian breeding lines and therefore any growth and agronomic characteristics will be within the range of conventionally developed canola cultivars, including hybrid varieties.
347. The introduced genes confer four phenotypic traits:
- tolerance to herbicide glufosinate ammonium through the PAT enzyme (all seven GM canola lines)
 - male sterility by expression of Barnase in the tapetum layer of developing flowers (MS lines only)
 - fertility restoration by expression of barstar in the developing flowers (RF lines, RFxMS hybrids)
 - resistance to aminoglycoside antibiotics such as kanamycin by expression of the NPTII protein (lines Topas 19/2, RF1, RF2, MS1 only)
348. The genetic modifications and expression of the respective genes are described in detail in Appendix 1.

349. The growth characteristics of each of the seven lines of GM canola in terms of phenology (eg. flowering period), pollen production and pollen viability (except in the male sterile lines), seed production, seed size, seed germination, dormancy and agronomic performance, including disease resistance potential and sensitivity to herbicides other than glufosinate ammonium, have been assessed as being within the range for conventionally developed canola varieties. Seed shattering ability, seed size and seed weight of glufosinate ammonium tolerant canola was no different to conventional canola lines indicating no alteration in the potential for seed dispersal (Canadian Food Inspection Agency 1995a; Canadian Food Inspection Agency 1995b; Canadian Food Inspection Agency 1996b; European Scientific Committee on Plants 1998a; USDA-APHIS 1998b; USDA-APHIS 1999b; USDA-APHIS 2002a; USDA-APHIS 2002b; USDA-APHIS 2002c; USDA-APHIS 2002d).

Glufosinate Ammonium Tolerant canola

350. Glufosinate ammonium tolerance is the most important trait when considering whether the genetic modification will have any impact on weediness of the GM canola lines because this trait provides for a possible selective advantage over non-tolerant canola.
351. A number of studies have investigated whether the introduction of glufosinate ammonium tolerance results in increased weediness. Four different glufosinate ammonium tolerant crops, oilseed rape, potato, maize and sugar beet were grown in 12 different habitats and monitored over a period of 10 years. In no case were the genetically modified plants found to be more invasive or more persistent than their conventional counterparts. Oilseed-rape expressing tolerance of the herbicide glufosinate, showed significantly lower seedling establishment when compared with conventional canola lines in six out of twelve cases and significantly higher in two cases. (Crawley et al. 2001). Poulsen et al. (1999) found no differences in competitive ability of glufosinate ammonium tolerant canola lines and non-transgenic cultivars grown either in monoculture or in mixture with barley. Transgenic lines only behaved differently from standard cultivars when glufosinate ammonium herbicide was applied.

Hybrid Breeding System

352. The male sterility and fertility restoration traits would not be expected to increase the weediness potential of canola. Cytoplasmic-male sterility is used widely in the conventional breeding of hybrid canola cultivars. The male sterility in the GM canola lines (MS1, MS8) is unlikely to increase the weediness potential any more so than would cytoplasmic male sterility. In fact, male sterility alone would provide a significant disadvantage to seed production and the persistence of male sterile canola in the field would be shortened where pollen from other sources is limiting.
353. Breeding for genotypic and phenotypic uniformity in crop plants, such as canola, can result in plants that are inbred. Inbred plants may display significant 'inbreeding depression', ie they have lowered fitness or vigour compared with their non-inbred or wild counterparts. In contrast, F1 hybrids from crosses of different inbred parental lines may exhibit hybrid vigour or heterosis, whereby they have increased vigour compared to parental lines (Allard 1999).
354. It is important to note that the hybrid vigour displayed in F1 RFxMS hybrids is not a function of the genetic modification, but is a result of the breeding of the two genetically distinct parents. The RF and MS genetic modifications provide a mechanism to allow controlled production of hybrid seeds which exhibit the natural

- phenomenon of hybrid vigour or heterosis. Hybrid vigour has the benefits of the production of a healthier plant, less influenced by disease and environmental conditions such as drought stress, and is most often measured in agronomic terms as increased yield. The degree of hybrid vigour achieved is related to the genetic background of the parental lines {Starmer, 1998 4483 /id /pt“eg ”}.
355. InVigor[®] canola hybrids have displayed yield increases of 10-20% over conventional open pollinated varieties in Australia and greater than 20% in Canada {Bayer CropScience, 2003 4551 /id;Clayton, 1999 4554 /id;Harker, 2003 4555 /id;Zand, 2002 4481 /id}. Data obtained in Australia indicate that the vigour exhibited by InVigor[®] canola hybrids falls within the range of vigour exhibited by conventional hybrid and open pollinated varieties of canola currently grown commercially (data supplied by Bayer, see Appendix 5 for further details).
 356. Hybrid vigour in InVigor[®] canola hybrids is manifested by superior seedling emergence and seedling vigour, greater uniformity and faster crop maturity and ripening, and by increased above ground biomass, pod numbers, pod size, seeds per pod, seed size, and increased quality parameters such as oil and protein {Bayer CropScience, 2003 4551 /id;Harker, 2003 4555 /id;Clayton, 1999 4554 /id /pt“information supplied by Bayer, ”}. Increased seed size has been correlated with increased vigour in canola hybrids {Butruille, 1999 4484 /id}.
 357. Increased seed numbers in InVigor hybrids might result in increased seed losses at harvest, and an increase in canola volunteers in subsequent seasons, however increased uniformity of crop maturation and ripening may result in reduced seed loss due to shattering prior to or during harvest {Bayer CropScience, 2003 4551 /id}. In a recent study of harvest losses in Canada, Gulden et al. {Gulden, 2003 3384 /id /d}reported that growers considered uneven crop maturity as a major factor in crop losses. Evidence from Canada also indicates that the greater crop health and competitiveness of hybrid canola, including InVigor canola can improve the suppression of weeds in-crop {Harker, 2003 4555 /id;Zand, 2002 4481 /id}.
 358. During production of F1 InVigor hybrid seed (eg certified seed) rows of the RF line are planted between rows of the MS line. The RF plants are mechanically removed (slashing/mulching) after flowering as only seed from the MS line is desired. If the RF rows were allowed to set seed prior to slashing this could result in an increase of the seedbank of canola and canola volunteers at the production site. Any weediness risk associated with such an occurrence is the same as that posed by planting the GM RF canola. The same consideration also applies to conventional hybrid seed production and can be effectively managed by good agricultural practice ensuring that slashing and mulching take place before seed set, or that any seed from the male line (ie RF) is removed.
 359. As noted above, the hybrid vigour manifested in InVigor canola crops (F1 generation) is not the result of single trait or locus. In general, hybrid vigour manifested in the F1 generation declines in subsequent generations {Falconer, 1996 1971 /id}. Therefore, although the F1 generation InVigor seed sown will exhibit hybrid vigour this will not result in increased weediness or invasiveness of F2 or subsequent generation seed.
 360. Fertility of plants from the fertility restorer line was reported by Bayer to be similar to the non-transformed parent, and these plants will not affect the male fertility of plants that lack the barnase gene (USDA-APHIS 1998b).

Antibiotic Resistance

361. The antibiotic resistance trait was used in the tissue culture (laboratory) stages of development of lines Topas 19/2, RF1, RF2 and MS1. There is no reason to consider that it would have any impact on the weediness of these GM canola lines and it will not be further considered in this Appendix. The nptII gene is not present in lines RF3 and MS8, the lines Bayer proposes to commercialise in Australia.

Other Attributes

362. Data provided by the applicant indicates that the GM canola lines developed for specific rainfall regions of Australia do not show any change in resistance or susceptibility to major canola pathogens such as blackleg compared to varieties that are conventionally bred to be grown in the same regions. Similarly, the GM canola lines not proposed for commercialisation (T45, Topas 19/2, RF1, RF2 and MS1) do not have altered disease characteristics. In addition, the application has provided data that indicate no differences between the GM canola lines and conventional canola have been observed during the numerous field trials conducted in Australia with respect to resistance or susceptibility to other canola pathogens or pests (eg. sclerotinia, flea beetles and diamondback moth larvae).
363. The applicant states that RF3 and MS8 hybrid canola lines did not exhibit any significant differences in susceptibility to temperature, humidity, desiccation, light or other environmental stress factors from those of other non-transformed canola cultivars during the period from planting to harvest.

AGRICULTURAL SYSTEMS

364. The majority of Australian farmers have moved away from aggressive tillage practices because of the extreme risk of soil erosion and adopted minimum or zero tillage methods (Sutherland 1999). Minimum tillage refers to the system of crop production where the soil is cultivated, or dug up, as little as possible, often only during the sowing process (zero tillage). This is in contrast to other cropping systems where the soil may be cultivated a number of times for seedbed preparation and/or to eliminate weeds before the crop seed is sown (Anon. 2001). Significant proportions of crops in Australia are seeded using zero-till methods (Sutherland 1999).
365. Since weeds are no longer controlled by non-selective tillage methods, crop sequences and seeding techniques are highly dependent on herbicides (Sutherland 1999). Non-selective herbicides, such as Roundup[®] (glyphosate) are used pre-sowing to control weeds. Many producers have moved from rotations including a pasture phase to continuous cropping practices with weed control becoming more dependent on selective herbicides and carried out in preceding crops (Table 1). The availability of non-GM herbicide-tolerant canola varieties has allowed in-crop control of weeds in areas where production was previously restricted. The introduction of triazine and imidazolinone tolerant canola (derived via conventional breeding) has allowed canola to be grown in areas where brassicaceous weeds are a problem. Reliance on herbicides in minimum tillage systems increases the likelihood of development of resistant weeds.

Table 1 Examples of typical cropping rotations in Australia.

Year	Example 1	Example 2	Example 3	Example 4	Example 5
1	Fallow	Pasture	Pasture	Canola	Pasture
2	Canola	Wheat	Canola	Wheat	Canola
3	Wheat	Canola	Wheat	Wheat	Wheat
4	Pasture/ Legume	Wheat	Wheat	Barley	Legume
5	Wheat/Barley	Pasture/ Legume	Legume	Fallow/ Legume	Wheat

366. In the US, Fawcett and Towery (2002) have reported a strong association between the use of herbicide-tolerant crops (GM and non-GM) and minimum tillage practices. The development of herbicide-tolerant crops has removed much of the uncertainty in weed control that prevented farmers from adopting minimum tillage techniques. In Western Canada, a survey of over 600 canola growers was conducted to determine the agronomic and economic impact of transgenic canola (Roundup Ready[®], Liberty Link[®] and InVigor[®] hybrids – the latter two canola varieties are glufosinate ammonium tolerant) (Serecon Management Consulting Inc & Koch Paul Associates 2001). Transgenic canola growers reported having made fewer tillage passes over their fields than growers of conventional varieties. The majority of growers planting transgenic varieties indicated that they utilise minimum or no till techniques for their operations.
367. A recent analysis by Norton {Norton, 2003 4116 /id /d} concluded that the adoption of GM herbicide tolerant canola varieties, such as InVigor[®] canola, in Australia could result in a significant increase in the use of minimum tillage in canola production.
368. In Canada, genetically modified glufosinate ammonium tolerant canola varieties (Liberty Link[®] and InVigor[®]) have been grown commercially since 1996. The uptake of the technology has been rapid, with 25% of the area sown to canola in Western Canada in 2002 being glufosinate tolerant GM canola. Only 15% sown was non-herbicide-tolerant canola and the remaining area was sown to other herbicide-tolerant canola cultivars, mainly GM glyphosate tolerant canola (R. Van Acker pers. comm. 2002).
369. Prior to the introduction of herbicide-tolerant canola, outcrossing between canola cultivars was of little concern to canola growers as all volunteers could be controlled by the application of the same herbicide. The education of farmers with regard to the introduction of herbicide-tolerant varieties in Canada and the implications for volunteer control was generally inadequate. This lack of information led to growers being concerned when volunteers in paddocks neighbouring herbicide-tolerant canola showed resistance to that herbicide, even though these volunteers could be readily controlled by the application of alternative herbicides.
370. It should be stressed that the occurrence of volunteer plants of a particular crop in seasons subsequent to its cultivation is a normal facet of agricultural production, and not in any way restricted to canola or GM crops. The control of volunteers in subsequent seasons is part of normal weed control operations and forms an integral part of agricultural production.

371. In U.K. trials, the number of glufosinate ammonium tolerant canola volunteers in the year following GM trials were comparable to or less than the number of conventional canola volunteers in the year following crops of conventional canola (Crawley et al. 1993; Sweet 1999). Information from commercial fields in Canada shows the same trend (MacDonald & Kuntz 2000). The incidence of glufosinate ammonium tolerant canola volunteers recorded in Bayer and OGTR monitoring reports at Australian release sites is consistent with the incidence of volunteers in the U.K., measuring from zero to several thousand (Norris et al. 1999; Salisbury 2002).
372. Salisbury (2002c) has noted that the incidence (germination rates) of volunteers at sites from previous Australian GM canola trials (glyphosate and glufosinate tolerant) sown in late spring or early summer is delayed and more variable than at sites sown in winter. Delayed germination of volunteers was more common from late spring/summer sown trials, with the majority of germination in year 2 and/or year 3 in 54 % of these trials. The reasons for this phenomenon are unclear, but one possibility is that higher temperatures at harvest may contribute to the development of secondary dormancy (J. Baker personal communication). In the U.K., the number of glufosinate ammonium tolerant volunteers following trials tended to be lower in the first year and more prevalent in the second year possibly due to post harvest conditions (Norris et al. 1999).
373. Analysis of monitoring reports from Australian GM canola trial sites indicate that at the majority (82.5 %) of winter sown GM trial sites no volunteers were recorded in the third year, while 17.5 % of sites still had small numbers of volunteers emerging in the third year (Salisbury 2002). Recent reports from OGTR monitoring indicate that the management practices and use of the sites after harvest of canola also affect persistence. The size and persistence of the seedbank can be influenced by machinery and conditions at harvest (Thomas 2000), cultivation practices following harvest (Lopez-Granados & Lutman 1998), soil type (Pekrun et al. 1998) and cultivar (Pekrun et al. 1997c; Lopez-Granados & Lutman 1998; Gulden et al. 2000).
374. Large numbers of viable conventional canola seed can persist in the seedbank for several years (Lutman 1993; Pekrun et al. 1998). This appears to apply equally to glufosinate ammonium tolerant canola. Large numbers of glufosinate ammonium tolerant canola seed persisted in the soil for up to three years after their release at some U.K sites (Norris et al. 1999). Similar results were obtained from trials in Denmark (Fredshavn & Poulsen 1996).
375. Bayer indicate that data collected from trials on the GM canola lines in Australia and overseas does not provide any evidence to suggest that the genetic modification results in other 'pleiotropic' effects that would increase the weediness of the canola plants. Herbicide tolerance is unlikely to confer any fitness advantage to volunteer GM canola and/or weedy relatives outside of the system where the herbicide is used.
376. Bayer made a parallel application to the APVMA for registration of glufosinate ammonium for use on InVigor[®] canola under the trade name Liberty[®]. The APVMA has registered glufosinate ammonium as Liberty[®] for use only in InVigor[®] canola crops. Glufosinate ammonium herbicide (or any herbicide with the same mode of action) is currently not registered by APVMA for any other use in broad acre agriculture.
377. Glufosinate ammonium is also registered in Australia as Basta[®] and Finale[®]. Finale[®] is used in non-crop agricultural areas, commercial and industrial areas and rights-of-way but is not a widely used chemical in these areas (Dignam 2001). In Australia

glufosinate ammonium is most widely used as Basta[®] for weed control in viticulture and horticultural crops. Tables 2 A and B show the percentage of horticultural Basta[®] use by state and by crop (information supplied by Bayer). These percentages do not represent the actual amounts of Basta[®] applied relative to other herbicides.

Table 2A. Horticultural Basta[®] use on a percentage basis by crop and State – up to 2001.

State	% of total horticultural Basta [®] use by state and by crop			
	Grapevines	Fruit trees ¹	Vegetables	Other
NSW	10.2	2.5	0	0
VIC	16.2	7.6	0	1.1
QLD	0.4	20.7	0.2	0
SA	32.2	1.8	0	0
WA	3.1	1.1	2.9	0
Total % of horticultural Basta [®] use	62.1	33.7	3.1	1.1

Information supplied by Bayer. 1: including bananas (about 20 %).

Table 2B. Horticultural Basta[®] use on a percentage basis by crop and State – current estimates.

State	% total horticultural Basta [®] use in by state and by crop		
	Grapevines	Fruit trees ¹	Other ²
NSW	13	2	0
VIC	16	2	0.5
QLD	0.5	24.5	0.5
SA	31	1	0
WA	5	1	0
TAS	1	1	0
Total % of horticultural Basta [®] use	66.5	32.5	1

Information supplied by Bayer. 1: including bananas (about 20%), 2: including strawberries and tomatoes

378. Normal management practices for the control of volunteers of conventional canola should be sufficient to control glufosinate ammonium tolerant canola volunteers. Table 3 shows the herbicide options that can be used to control Brassica weeds, including canola volunteers in a range of cropping situations. The genetic modification only confers tolerance to glufosinate ammonium and field observations by Bayer confirm that their GM canola lines are still susceptible to other herbicides that control canola and related weedy species (eg. glyphosate, phenoxy and sulfonylureas). GM herbicide-tolerant canola, including glufosinate ammonium tolerant canola, did not lead to increased problems of volunteer management in subsequent crops in the UK (Norris et al. 1999). In its InVigor[®] Canola Crop Management Plan, Bayer recommends that when spraying for volunteer canola, growers are to be aware of previous herbicide-tolerant cropping in the vicinity and to make their herbicide choice appropriately. Growers are encouraged to communicate with adjoining land-owners regarding the use of genetically modified varieties and cropping rotations.

Table 3 Herbicides for control of Brassica weeds in crop and fallow.

Herbicide	Group	Rate/ha	Situation
Chlorosulfuron	B (ALS inhibitor)	15-20g/ha	Wheat, (barley and oats post emergence)
Metsulfuron	B (ALS inhibitor)	5-7 g/ha	wheat, triticale, barley, fallow
Metsulfuron + thifensulfuron	B (ALS inhibitor)	30-35g/ha	wheat, barley
Flumetsulam	B (ALS inhibitor)	15-25g/ha	wheat, barley, oats, lupins
Triasulfuron	B (ALS inhibitor)	30-35g/ha	wheat (pre only)
Tribenuron	B (ALS inhibitor)	20-25g/ha	fallow
Metosulam	B (ALS inhibitor)	5-7g/ha	wheat, barley, oats, lupins
Imazamethapyr	B (ALS inhibitor)	0.2-0.3L/ha	field pea, faba bean
Triasulfuron + terbutryn	B (ALS inhibitor) + C	250-500g/ha	wheat, barley
Cyanazine	C (triazine)	3 or 4L/ha	chickpea, field pea, faba bean
Metribuzin	C (triazine)	0.435-0.58L/ha	chickpea, field pea, faba bean
Simazine + prometryn	C (triazine)	1.5+1.5L/ha	chickpea
Terbutryn	C (triazine)	0.85-1.1L/ha	oats
Simazine	C (triazine)	0.8-2L/ha	lupins, chickpea, faba beans, lentil, TT canola
Atrazine	C (triazine)		TT canola. Sorghum, maize, fallow
Simazine + imazathapyr	C (triazine) + B (ALS inhibitor)		chickpea
Simazine + diflufenican	C (triazine) + F		lupins
Diflufenican	F (Inhibitors of carotenoid biosynthesis)	0.15-0.2L/ha	field pea, lupins
Diflufenican+ MCPA	F (Inhibitor of carotenoid synthesis)+I	0.5-1.0	wheat, barley, oats
Diflufenican + bromoxynil	F + C	0.5-1.0	wheat, barley
2,4-D amine	I (phenoxy)	0.7-2.1L/ha	wheat, barley, oats, fallow
2,4-D IPA	I (phenoxy)	0.8-1.6L/ha	fallow
2,4-D ester	I (phenoxy)	0.35-0.7L/ha	wheat, barley, fallow
MCPA amine	I (phenoxy)	0.35-1.6L/ha	wheat, barley, oats, field pea
MCPA LVE	I (phenoxy)	0.5-1.6L/ha	wheat, barley, oats
2,4-DB	I (phenoxy)	2.1-3.2L/ha	wheat, barley, oats, lucerne. Medics
Diuron	I (urea)	0.9L/ha	oats
Diuron+MCPA	I (urea) + I (phenoxy)	0.28 + 0.5	wheat, barley
Paraquat + diquat	L (bipyridil)	1.6-2.4L/ha	fallow
Glyphosate	M (Inhibitor of EPSP synthase)	1.5-9L/ha	fallow

379. Glufosinate ammonium is used extensively in vineyards to control a wide range of broadleaf and grass weeds {Bayer CropScience, 2002 4556 /id /ft“, information supplied by Bayer”}. Canola or *Brassica juncea* are sometimes sown in vineyards for biofumigation. Biofumigation refers to the suppression of soil-borne pests and pathogens by biocidal compounds (isothiocyanates) released in soil when glucosinolates in Brassica green manure or rotation crops are hydrolysed {Kirkegaard, 1998 4588 /id}. In Vigor[®] canola would not be controlled by Basta[®]. However, it would be controlled by other management options. While this would not present a risk to human health and safety or the environment, Bayer’s InVigor[®] Canola Crop Management Plan recommends that InVigor[®] canola not be grown in vineyards {Bayer CropScience, 2002 4557 /id}. . Table 4 shows the herbicide options that can be used to control Brassica weeds, including canola volunteers, in vineyards.

Table 4 Herbicides for control of Brassica weeds in vineyards.

Active Ingredient	Group	Trade Name
Simazine	B	various
Diuron	C	various
Oryzalin	D	Surflan [®]
Amitrole & ammonium thiocyanate	F	Amitrole [®] T
Norflurazon	F	Solicam [®]
Oxyfluorfen	G	Goal [®]
Dichlobenil	K	Casoron [®]
Paraquat + diquat	L	Spray Seed/Tryquat [®]
Glyphosate	M	Roundup [®]
Glyphosate-trimesium	M	Touchdown [®]

380. Herbicides are not the only tools to manage conventional and GM glufosinate ammonium tolerant volunteer canola. In the InVigor[®] Canola Crop Management Plan submitted by Bayer, a number of recommendations are made to growers to prevent persistence and spread of glufosinate tolerant In Vigor[®] canola. They recommend that growers:

- optimise harvest timing to minimise pod shattering to prevent seed loss*;
- adjust harvester settings to optimise harvesting efficiency and minimise seed shedding*;
- preferably harvest GM crops after non-GM crops to minimise seed transfer;
- use equipment that has been thoroughly cleaned;
- avoid spillage during transport*;
- store InVigor[®] canola seed separately and label clearly;
- use newly produced certified seed; and
- maximise germination of lost canola seeds and do not use deep soil inversion as the first cultivation after harvest.

*Note that these practices also contribute to attaining maximum yield.

381. The development of seedbanks of transgenic glufosinate ammonium tolerant canola (and of non-GM canola) can be reduced by delaying cultivation (leaving seed on soil surface) and a shallow soil cultivation to avoid burial of seed (Pekrun et al. 1998; Gulden et al. 2000). To reduce seedbank levels, shallow cultivation can be used to stimulate germination and emergence of buried seeds (Legere et al. 2001). Practices such as cultivation are effective in killing emerged plants (Legere et al. 2001). Although cultivation is an effective weed management tool, there is no a priori expectation that the use of InVigor[®] canola would lead to increased cultivation. A recent analysis by Norton (2003) concluded that “the proposed use patterns for both InVigor[®] canola and Roundup Ready[®] canola would allow growers to reduce tillage operations before sowing and rely on post emergence weed control in-crop.”

382. During field trials of GM canola in Tasmania, a number of trial sites developed persistent seedbanks due to deep cultivation and burial of seed causing secondary seed dormancy. Burial of non-GM canola will also lead to secondary seed dormancy and the genetic modifications do not make the seed more dormant or persistent. However, maximal containment of releases at the field trial stage is part of a precautionary approach adopted until a comprehensive risk assessment, such as this current assessment, establishes that there are no risks from persistence of the GM canola. Therefore extra requirements were imposed on the field trials to limit the persistence of the GM canola.

383. As the seven GM canola lines will be no more invasive or persistent than conventional canola and can be controlled by the same herbicide and cultural practices currently used to control volunteer canola no specific management conditions are required for this release.

Dissemination of seed by animals

384. It is conceivable that small amounts of seed could be dispersed in the faeces of grazing livestock.
385. Small amounts of canola seed may also be dispersed via the faeces of animals such as livestock. An Australian feeding study found that germinable canola seed was excreted from sheep for 5 days after it was last included in the diet {Stanton, 2003 4525 /id}. The percentage of germinable seed excreted daily was 0.1 % of the average daily intake. However, only 1-1.5 % of canola seed ingested by sheep was excreted whole. The germination rate was approximately 40 % for seed passed in faeces on the first day but declined to less than 10 % for seed passed in faeces after the first day of excretion. As with any other crop, if such low levels posed a marketing concern, isolating livestock from designated areas for 7 to 10 days would ensure that all viable canola seeds would be passed before stock were moved away from the paddock. Furthermore, in the majority of cases, canola used in stockfeed is the high protein meal that remains after crushing the seed for oil extraction. In these circumstances no viable canola seeds would be present following crushing.
386. To prevent the possible dispersal of viable glufosinate tolerant canola seed in the faeces of stock grazing on InVigor[®] canola stubble, Bayer recommends that livestock be held within a single grazing area for a period of at least 7 to 10 days {Bayer CropScience, 2002 4557 /id}.
387. The possibility of dissemination of canola seed by wild birds consuming seed directly from the crop or in the manure of barn produced poultry fed whole canola seed has been raised. Birds such as cockatoos and sparrows can shred or remove pods during development and at maturity (Stanley & Marcroft 1999). Canola is soft-seeded and is very unlikely to survive passage through the gut of a bird. While no direct experimental data is available to assess the likelihood of dispersal of viable canola seed by wild birds, there is no evidence that glufosinate tolerant canola is more likely to be consumed by birds than conventional canola. Growers in some areas of Australia apply poultry manure from poultry farming operations to fields as fertiliser, however no incidences of weed problems resulting from the application of manure have been reported.
388. As noted previously, the seed shattering ability, seed size and seed weight of the seven GM canola lines are no different to conventional canola indicating no alteration in the potential for seed dispersal as a result of the genetic modifications. InVigor[®] hybrid canola display hybrid vigour resulting in superior seedling emergence and seedling vigour, and increased seed numbers and seed size. However this vigour is not the result of the genetic modifications and is within the range of vigour shown by conventional hybrids.
389. Dissemination of herbicide-tolerant or herbicide-susceptible conventional canola by birds or other animals has not resulted in any significant dispersal. It is therefore unlikely that this will be a significant means of dissemination of glufosinate ammonium tolerant canola. The genetic modifications will not make the GM glufosinate

ammonium canola more invasive than conventional canola, and the available field observations support the conclusion that the main and most important means of dispersal of canola are via human activities such as sowing, harvesting and transport, and handling pre- and post-harvest.

390. In addition, any seed dispersed by birds or other animals would not represent an environmental risk because the genetic modifications will not make the GM canola lines more invasive or persistent than conventional canola and therefore no risk management conditions are required. The GM canola lines can be controlled by the same herbicide and cultural practices currently used to control volunteer canola.

UNCROPPED DISTURBED HABITATS

391. Due to its primary colonising nature, canola can take advantage of disturbed land (Salisbury 2002), however, canola is a poor competitor and will be displaced unless the habitats are disturbed on a regular basis (Organisation for Economic Co-operation and Development (OECD) 1997a; Beckie et al. 2001). There appears to be no evidence that the presence of herbicide-tolerant transgenes would greatly influence the ability of plants to survive in a feral environment (Wilkinson et al. 1995) except in the presence of the specific herbicide. Glufosinate ammonium tolerant canola does not show any enhanced stress adaptation relative to the conventional counterpart, other than tolerance to the herbicide (Canadian Food Inspection Agency 1995b).
392. Bayer's monitoring results from unmanaged areas adjacent to fields and along transportation routes in Canada indicate that the frequency of GM herbicide-tolerant canola volunteers is equal to conventional volunteers. Both are equally likely to appear by the roadside if seed falls from trucks or farming equipment (Rasche & Gadsby 1997; MacDonald & Kuntz 2000). Several different types of canola were identified in these areas with the distribution most likely influenced by the selection of which cultivars local farmers choose to cultivate (MacDonald & Kuntz 2000).
393. In Canada and France, populations of volunteer canola are often prevented from reaching maturity by mowing or herbicide application (MacDonald & Kuntz 2000; Pessel et al. 2001). In Scotland, populations of feral canola were not eliminated entirely by mowing, herbicide application or a combination of both, with survival due to plants being missed during control operations (Wilkinson et al. 1995).
394. As previously noted, a recent survey of roadsides in the major canola growing regions of Australia found that in most cases canola plants were growing within 5 m of the roadsides, apart from plants observed along railway tracks and sidings (Agrisearch 2001). In a survey of local councils and road and rail authorities, 30 % of councils and 4 % of road and rail authorities reported canola as a weed, when prompted. Of those reporting canola, approximately 70 % did nothing to control it.
395. There is no evidence that the presence of herbicide tolerance transgenes would influence the ability of plants to survive in these disturbed environments except in the presence of glufosinate ammonium. Although glufosinate ammonium is registered for use in commercial and industrial areas, rights-of-way and other non-agricultural areas under the trade name Finale[®], it is not widely used for weed control by local councils and Road and Rail authorities (Dignam 2001). A report from Agriculture Western Australia (Anon. 2001) states that shire councils rely on herbicide mixtures for effective control of roadside weeds. Glyphosate was reported as the herbicide of choice for chemical control by Road and Rail authorities and Local Councils, but no data was presented in this study on the extent to which herbicide mixes are used (Dignam 2001).

396. In their InVigor® Canola Crop Management Plan, Bayer emphasises the need to control volunteer canola populations along fence lines and roadsides. Bayer recommends avoiding spillage during transport and intermediate storage, both on and off the farm, and minimising and eliminating volunteer populations prior to flowering and seed set. It should be noted that this is a standard and accepted practice for controlling weed numbers. Where a farmer grows a herbicide-tolerant crop along a boundary fence line that is adjacent to a neighbouring canola crop, Bayer also recommends that the farmer notify the adjoining land owner.
397. The seven GM canola lines will be no more invasive or persistent than conventional canola and do not represent a risk to uncropped disturbed environments and therefore no risk management conditions are required. The GM canola lines can be controlled by the same herbicide and cultural practices currently used to control volunteer canola.

UNDISTURBED NATURAL HABITATS

398. Canola having been bred as a cultivated crop can only germinate and establish under optimal growing conditions within a well managed agronomic system. These conditions are not generally available in non-cultivated areas. GM herbicide-tolerant canola has no altered invasive potential which would enhance its weedy potential in natural habitats (Canadian Food Inspection Agency 1995b; Rasche & Gadsby 1997; MacDonald & Kuntz 2000).
399. The potential weediness of glufosinate ammonium tolerant canola has been investigated in a long-term ecological study conducted at 12 sites in 8 different habitats over a 10 year period in the U.K. (Crawley et al. 1993; Crawley et al. 2001). Sites were monitored annually to follow the fate of sown individuals, to measure recruitment onto unsown areas nearby and to determine whether there was any resurgence following natural disturbance in later years. In six out of 12 sites, seedling establishment in the first year was significantly lower for GM canola than for conventional canola. The genetic alterations to glufosinate ammonium tolerant canola did not appear to result in weedy characteristics as no population of canola, either conventional or GM, persisted beyond the second year. None of the crops, conventional or transgenic, increased in abundance at any of the sites. The results showed that transgenic glufosinate ammonium tolerant canola was no more invasive or persistent than its conventional counterpart. Work by Norris et al. (1999) in the U.K. also concluded that GM herbicide-tolerant canola varieties, including glufosinate ammonium tolerant canola, are no more persistent or invasive than conventional types.
400. A survey by Dignam (2001), reported that canola was not present in any National Parks in the major canola growing areas of Australia.
401. The seven GM canola lines will be no more invasive or persistent than conventional canola and do not represent a risk to undisturbed environments and therefore no risk management conditions are required. The GM canola lines can be controlled by the same herbicide and cultural practices currently used to control volunteer canola.

SECTION 3 CONCLUSIONS REGARDING WEEDINESS

402. Canola is not a significant weed in habitats outside agricultural areas and does not pose a serious threat to the environment and biodiversity. Conventional canola can persist as an agricultural weed, particularly as volunteers following canola crops. It is spread via

human activities such as sowing, harvesting, transport, and handling pre- and post-harvest. It shares some life history characteristics with other weeds but is a poor competitor and is not invasive. It does not invade Australian native habitats and is usually present only in disturbed habitats adjacent to farms and vacant habitats.

403. The introduced genes do not increase the potential weediness of the GM canola lines or provide these plants with an ecological advantage over conventional canola except in the presence of glufosinate ammonium. The germination, seed dormancy and fitness traits such as herbicide sensitivity, disease resistance, stress adaptation and competitiveness for the seven GM canola lines fall within the range of conventionally bred canola varieties.
404. InVigor[®] canola hybrids derived from crossing RF and MS lines display superior seedling emergence and vigour, and increased seed yield and size compared to the parent RF and MS lines. However, these and other life history characteristics are within the range exhibited by conventional hybrids and open pollinated canola. The hybrid vigour is not a direct result of the genetic modifications, but the male sterile (MS) and fertility restorer (RF) lines provide a means of ensuring hybrid seed.
405. The GM canola lines do not have any competitive advantage in the absence of glufosinate ammonium and their susceptibility to other herbicides is no different to conventional canola.
406. The APVMA has registered glufosinate ammonium as Liberty[®] for use only in InVigor[®] canola crops. Glufosinate ammonium herbicide is currently not registered by APVMA for any other use in broad acre agriculture. Glufosinate ammonium is also used in horticulture and viticulture (registered as Basta[®]) and non-crop agricultural areas, commercial and industrial areas and rights-of-way (registered as Finale[®]) but is not a widely used chemical for this use.
407. The glufosinate ammonium tolerant GM canola lines can be managed and controlled in the same manner as conventional canola volunteers using other herbicides and non-chemical management techniques.
408. In summary:
 - The risk of the GM canola lines being a weed in agricultural environments is not likely to be greater than for conventional canola.
 - The risk of the GM canola lines becoming a weed in non-cropped disturbed environments is not likely to be greater than for conventional canola.
 - The risk of the GM canola lines being invasive and spreading into undisturbed environments is not likely to be greater than for conventional canola.
 - As the risk that the GM canola lines will be more likely than conventional (non-GM) canola to spread in the environment, and result in more detrimental environmental impact is negligible, no management conditions are required.

APPENDIX 5 ENVIRONMENTAL SAFETY — TRANSFER OF INTRODUCED GENES TO OTHER ORGANISMS

409. Under section 51 of the Gene Technology Act 2000, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. This part of the document considers potential hazards that may be posed to the environment. In this context, the potential for gene transfer from the GMO to other organisms was considered.
410. In general terms, the types of hazards that might result from transfer of the genes introduced into the GM canola lines T45, Topas19/2, RF1, RF2, RF3, MS1 and MS8 to other organisms could include the production of herbicide-tolerant weeds, some of which may have the potential to compete with native flora thereby reducing biodiversity.
411. When analysing the risk of gene flow (transfer), a distinction needs to be made between hybridisation and introgression. Hybridisation is the crossing between two different plants, either of the same or different species, resulting in the production of hybrid progeny. Progeny derived from crosses between plants of different species or genera are known as inter-specific hybrids. In the consideration of gene transfer between species inter-specific hybrids are often simply referred to as hybrids. Introgression is the incorporation of a gene or genes into the population after a hybridisation event.
412. A number of factors influence the likelihood of gene flow occurring. Pre-fertilisation considerations include physical proximity and pollen movement, synchrony of flowering, breeding system and floral characteristics and competitiveness of pollen. Post-fertilisation considerations include sexual compatibility, hybrid viability and fertility, viability and fertility of progeny through several generations of backcrossing and successful incorporation of the modified genes into the genome (introgression). For successful gene transfer to occur, all pre- and post-fertilisation requirements must be met. Failure to meet any one requirement will mean that gene transfer and introgression cannot occur.
413. The potential hazards are addressed in the following sections, with respect specifically to:
- other canola plants (Section 1 of this Appendix);
 - other plants (Section 2 of this Appendix); and
 - other organisms (Section 3 of this Appendix).

SECTION 1 TRANSFER OF INTRODUCED GENES TO OTHER CANOLA PLANTS

414. This section will focus on the likelihood of gene flow (transfer) and introgression from the GM canola lines to other canola crops and make conclusions about the consequences of these risks for the environment.

Section 1.1 Nature of the gene transfer hazard

415. Bayer are seeking regulatory approval for seven (7) genetically modified lines of canola: T45, Topas19/2, MS1, RF1, RF2, RF3 and MS8. Lines MS1, MS8, RF1, RF2 and RF3 and hybrids derived from MS x RF crosses are covered by the registered trade name InVigor[®] canola. All seven of the GM canola lines have been genetically

modified to introduce tolerance to the herbicide glufosinate ammonium. Five of the GM canola lines, RF1, RF2, RF3, MS1 and MS8, have been modified to introduce a novel hybrid breeding system, based on genetically modified male sterile (MS) and fertility restorer (RF) lines. Four of the seven lines (Topas19/2, RF1, RF2 and MS1) have also been modified to introduce an antibiotic resistance marker gene (see Appendix 1 for details).

416. Bayer has indicated that it only intends the commercial release of InVigor[®] canola lines RF3 and MS8 in Australia, but is seeking approval for all seven lines to achieve consistency with existing Australian and overseas regulatory approvals.
417. All seven GM canola lines were comprehensively considered with respect to gene transfer however, given the fact that only RF3 and MS8 lines are intended for commercialisation in Australia, particular attention is given to these lines.
418. Transfer of the introduced genes to other canola plants would present the same hazards and have the same potential environmental impacts as the presence of the genes in the GM canola.
419. If transfer occurred to canola crops tolerant to other herbicides this might present different risks regarding weediness and increase the possibility that the genes could spread in the environment.

Section 1.2 Likelihood of the gene transfer hazard occurring

1.2.1 Outcrossing within canola

420. As there are no sexual barriers to outcrossing, cross-pollination between non-GM herbicide susceptible, non-GM herbicide tolerant and GM herbicide tolerant canola crops is inevitable given sufficient proximity and exposure. There is no indication that the genetic modification per se affects the rate of outcrossing, therefore the results of studies on outcrossing rates between conventional canola apply equally to genetically modified glufosinate ammonium-tolerant canola. Many studies on pollen flow use herbicide tolerance genes as markers with hybrids resulting from outcrossing events identified by the presence of herbicide tolerance in non-herbicide tolerant crops, or multiple herbicide tolerant types in single herbicide tolerant crops (Salisbury 2002).
421. Canola is mainly self-pollinating though it is estimated that outcrossing occurs at approximately 30% (ranging between 12 and 47 %) in adjacent plants (Williams et al. 1986; Becker et al. 1992). The highest rate of cross-pollination requires close proximity and occurs in situations where there is physical contact with neighbouring plants, although pollen can be transferred over longer distances by insects and wind. In general, wind-borne pollen plays a minor role in long distance pollination with the vast majority of pollen travelling less than 10 metres (m). See review “Biology and Ecology of Canola (*Brassica napus*)” (2002), available at the OGTR website (www.ogtr.gov.au) for more detail on pollination in canola.
422. In Australia, honey bees (*Apis mellifera*) are believed to be the main insect responsible for transfer of canola pollen over long distances. The majority of pollen collected by *A. mellifera* is transferred less than 5 m but bee flights have been measured at distances of 1 to 2 km, and even up to 4 km (for more detail refer to OGTR 2002a).
423. Populations of bumblebees (*Bombus terrestris*) are also present in Tasmania. Bumblebees were first observed in Tasmania in 1992 and are distributed mainly in the southern areas of Tasmania but some sightings have been confirmed in northern areas

(Buttermore & Hergstrom 2000). Although bumblebees tend to forage at greater distances than honey bees, pollen is generally deposited on neighbouring plants (Cresswell et al. 1995). In a German study, a high proportion of bumblebee workers were found to forage between 600 and 1750m from the nest (Walther-Hellwig & Frankl 2000) but have been observed foraging at distances up to 3.2 km from the nest (R. Frankl pers. comm.). There is no difference in the amount of pollen transferred by each bee species (Cresswell et al. 1995).

424. There is no reason to expect that the genetic modifications in any of the GM canola lines (T45, Topas19/2, MS1, RF1, RF2, RF3 and MS8) will increase the likelihood of outcrossing to other plants compared to non-GM canola. Bayer has reported that the nectaries in the flowers of the seven GM canola lines develop normally and insect activity was also normal. GM canola lines MS1 and MS8 lack anthers and do not produce pollen. Pollen production in the other GM canola lines is no different to non-GM canola. Hybrid plants resulting from MSxRF crosses also have normal flower morphology, fertility, and attractiveness to insect pollinators and normal insect activity was observed on all these plants (USDA-APHIS 1999).
425. In the broad acre field situation, cross pollination between the GM canola lines and other canola would be most likely to occur when canola crops are grown in adjacent paddocks and flower synchronously and where there is minimal separation distance between the two crops. Cross pollination is also likely where volunteer plants emerge and develop to flowering stage after canola crops are harvested or where feral canola populations resulting from seed being carried off-farm establish along roadsides adjacent to cropping land where canola is planted.
426. Differences in outcrossing rates reported in the scientific literature are likely to be due to differences in cultivars used, experimental design, differences in the size of pollen source and recipient crops and their spatial arrangement, local topography and environmental conditions (Eastham & Sweet 2002). Downey (1999b) reported that outcrossing between large commercial fields in Canada was substantially lower than that previously observed in experiments between large commercial fields and small plots (Stringam & Downey 1982). However, in a comparison by Salisbury (2002b) of outcrossing rates at similar distances from small plot trials and large field trials, outcrossing rates in large field trials tended to be somewhat higher.
427. In male sterile plants there is no competition from endogenous pollen, which in fully fertile plants may significantly out-compete pollen from another source. Male sterile lines will be pollinated by foreign pollen and outcross with neighbouring fully fertile conventional canola at higher frequencies and at greater distances than traditional varieties (Simpson et al. 1999). Male sterile or emasculated bait plants have been used to detect outcrossing at distances up to 4 km from the pollen source (Simpson et al. 1999; Thompson et al. 1999). Studies using male sterile or emasculated bait plants only give an indication of the potential for outcrossing and not the likelihood of outcrossing actually occurring (Salisbury 2002).

Outcrossing rates in the Northern Hemisphere

428. Overseas studies have shown that the frequency of outcrossing varies with distance, but in general, outcrossing rates at 50 m from the source field and beyond are significantly less than 1 % (unless male sterile or emasculated plants were used in the study). As noted above, canola is mostly self-pollinating, but where male sterile plants are used as the pollen recipient and as indicator of pollination and subsequent seed set, the level of

cross-pollination will be an overestimate. Studies conducted in large fields with fertile canola, outcrossing rates of 1.1 to 3.3 % have been measured at distances up to 5 m from the source field (eg Champolivier et al. 1999; Beckie et al. 2001). At distances up to 50 m, outcrossing rates below 0.4 % have been measured (eg Champolivier et al. 1999; Downey 1999a; Downey 1999b; Beckie et al. 2001; Norris unpublished, cited in Eastham & Sweet 2002). Outcrossing rates of 0.15 % (Beckie et al. 2001), 0.1 and 0.4% (Downey 1999a; Downey 1999b), and 0.5 and 0.25 % (Norris unpublished, cited in Eastham & Sweet 2002) have been measured up to 100 m. Outcrossing rates below 0.1 % were measured up to 250 m from the source field (Norris unpublished, cited in Eastham & Sweet 2002).

429. Studies of outcrossing rates between GM glufosinate ammonium-tolerant canola and conventional canola at trial sites in the U.K. have found that the frequency of glufosinate ammonium tolerant outcrossing decreased with increasing distance from the source of GM glufosinate ammonium tolerant canola (Simpson et al. 1999; Snow et al. 1999; Ingram 2000; Norris & Sweet 2003). At one site, frequencies of outcrossing ranged from 2 % at 4 m to 0.05 % at 56 m from the pollen source (Simpson et al. 1999). Similar levels were detected by Norris and Sweet (2002), however, at one of the sites studied, some long-distance outcrossing events were detected. The authors cited a number of factors that may have influenced these results including the contamination of the seed lot with male sterile or herbicide tolerant seeds, disturbance of insect or air currents by stands of trees or the invasion of the field by demonstrators during the flowering period.

Outcrossing rates in Australia

430. In 2000, an Australian study determined outcrossing rates between commercial fields of non-GM canola with tolerance to the herbicide OnDuty[®] (an imidazolinone herbicide) and conventional canola (Rieger et al. 2002). This was possible because the herbicide tolerant variety was released commercially in Australia for the first time in 2000. Fields in New South Wales, Victoria and South Australia, representing a diverse range of environments, were sampled. In each of the 63 fields tested, 10 samples were collected from three locations at varying distances from the pollen source. The seed was planted in an irrigated field along with two resistant and two susceptible cultivars.
431. To determine whether pollen mediated gene flow from source to sink fields had occurred, the seedlings were screened with the herbicide. Only 30% of samples screened revealed herbicide-resistant individuals and resistance frequencies varied up to a maximum of 0.197%. When individual samples were pooled within these fields, resistance was evident in 63% of these fields, although only a few had more than 0.03% resistance. The highest frequency of resistance on a paddock basis 0.07%. The results indicate that gene flow via pollen movement occurs between canola fields. However, even adjacent commercial canola fields in Australia will have much less than 1% gene flow (Rieger et al. 2002).
432. Previous studies have reported cross-pollination at higher frequencies close to the source field, with rates declining further from the pollen source (eg Scheffler et al. 1993; Staniland et al. 2000). In contrast, Rieger et al. (2002) found that comparison of samples within a field did not demonstrate a consistent edge effect. In fields where the edge closest to the pollen source was less than 100m, similar frequencies of resistance were found at all three sample points within the field. Although some fields did show a decline in resistant individuals with distance from the edge of the field, the majority of

fields, particularly those further from the source field, were more variable (Rieger et al. 2002).

1.2.2 Transfer of genes between MS x RF hybrids and conventional canola

HYBRID VIGOUR

433. Traditional plant breeding selects for plants with valuable agronomic characteristics such as oil content or disease resistance, but in plants such as canola, the inbreeding process produces parental plants with significant inbreeding depression. These plants have lowered fitness or vigour compared with their non-inbred or wild counterparts. F₁ hybrids from crosses of inbred parental lines may exhibit hybrid vigour, whereby they have increased vigour compared to parental lines (Allard 1999). In most cases the resultant increase in vigour is measured as increased yield. This increase in vigour is greatest in the F₁ population and declines in subsequent generations.
434. The hybrid vigour observed in InVigor[®] hybrid canola is not a direct result of the genetic modification, ie it is not encoded by a 'gene construct' that can be transferred to other plants as a single locus in the same way as the herbicide tolerance gene. The MS and RF genetic modifications create a breeding system in which hybrid progeny are assured because the male sterile lines are obligate outcrossers and can only produce hybrid seed. Hybrid vigour results from an increase in heterozygosity in first (F₁) generation crosses between lines and can affect characters such as seed size and other seed parameters, time of flowering and plant growth rate. In general, hybrid vigour manifested in the F₁ generation declines in subsequent generations (Falconer & Mackay 1996).
435. InVigor[®] canola hybrids have displayed yield increases of 10-20% over conventional open pollinated varieties in Australia and greater than 20% in Canada (Clayton et al. 1999; Zand & Beckie 2002; Bayer CropScience 2003; Harker et al. 2003). Hybrid vigour in InVigor[®] canola hybrids is manifested by superior seedling emergence and seedling vigour, greater uniformity and faster crop maturity and ripening, and by increased above ground biomass, pod numbers, pod size, seeds per pod, seed size, and increased quality parameters such as oil and protein (information supplied by Bayer, Clayton et al. 1999; Bayer CropScience 2003; Harker et al. 2003). However Australian data indicate that the enhanced agronomic performance exhibited by InVigor[®] hybrids falls within the range of vigour exhibited by conventional hybrid and open pollinated (inbred) varieties of canola currently grown commercially. InVigor[®] hybrid canola displayed approximately 15 % greater vigour than a conventional open pollinated variety, but 20 % less vigour than a conventional hybrid variety (data supplied by Bayer).
436. Because hybrid vigour declines in subsequent generations, the hybrid vigour displayed by the progeny of InVigor[®] hybrids (F₂ generation) will be less than that of the initial hybrids (the F₁ generation). It is therefore likely that there would only be a small difference between the vigour displayed by InVigor[®] F₂ progeny and the parental varieties. In general, hybrid vigour displayed in F₁ crop hybrids tends to decline in subsequent generations (Falconer & Mackay 1996).
437. Any transfer of the barnase gene to other canola plants will not have any negative environmental impacts because it will only result in male sterility and not confer any selective advantage in terms of weediness or persistence. However, since male sterility increases the likelihood of being pollinated by external sources (Lefol et al. 1991;

- Thompson et al. 1999), male sterile plants would have a marginally higher probability of acquiring genes from other plants. However, 50 % of the progeny of such crosses would be male sterile which, unless pollinated, cannot reproduce and so are unlikely to persist in the environment. The remaining 50 % of the progeny will be non-transgenic
438. If crossing of the fertility restorer line of InVigor[®] canola (RF1, RF2 and RF3, homozygous for both the bar and barstar genes) and conventional canola did occur, 100 % of progeny would be hemizygous for both the glufosinate ammonium tolerance bar gene and fertility restorer barstar gene. Backcrossing of hemizygous progeny with non-InVigor[®] canola over subsequent generations would, in the absence of selective pressure (glufosinate ammonium application), lead to a decrease in the presence of transgenes in the population. However, given that plants resulting from a cross are self-fertile, between 53 and 88 % of flowers will be self-pollinated resulting in plants homozygous for both the bar and barstar genes.
 439. Crosses of the male sterile line of InVigor[®] canola (MS1 and MS8, hemizygous for both the bar and barnase genes) with conventional canola would result in 50 % of plants with no transgenes (fertile and herbicide susceptible) and 50 % of plants that are male sterile and glufosinate ammonium-tolerant . Backcrossing of male sterile, glufosinate ammonium-tolerant progeny (these plants are unable to self-pollinate) with non-InVigor[®] canola over subsequent generations would, in the absence of selective pressure (glufosinate ammonium application), lead to a decrease in the presence of transgenes in the population.
 440. In the context of commercial broadacre production of InVigor[®] canola, it would be the hybrid canola seed resulting from crosses of the RF3 and MS8 parental lines that would be distributed to growers for sowing. This seed consists of two genotypes in equal proportions. One genotype will be hemizygous for the MS (barnase gene), hemizygous for the RF (barstar gene) and have two hemizygous copies of the glufosinate ammonium tolerance trait (bar gene) ($MS_1wt_1RF_2wt_2$ – remembering that the bar gene is linked to both the MS and RF gene). The second genotype does not contain the MS (barnase gene) and is hemizygous for the RF (barstar gene) and the glufosinate ammonium tolerance gene (bar gene) ($wt_1wt_1RF_2wt_2$).
 441. Mendelian inheritance dictates that crosses between InVigor[®] hybrid canola and a wild type (eg. conventional canola) will, on average, result in 62.5 % of plants that contain the glufosinate ammonium tolerance gene (bar gene), including 12.5 % of plants that are male sterile and herbicide tolerant (Table 1). The remaining 37.5 % will contain no transgenes.

Table 1: Average proportion of each type of plant expected to be produced in a cross between InVigor[®] hybrid canola and non-InVigor[®] canola (where wt_1 represents conventional canola).

Type	Genotype	Genotype (%)	Phenotype	Phenotype (%)
1	$wt_1wt_1wt_2wt_2$	37.5	Fertile Herbicide susceptible No transgenes	37.5
2	$wt_1wt_1wt_2RF_2$	37.5	Fertile Herbicide tolerant	50
3	$MS_1wt_1wt_2RF_2$	12.5		
4	$MS_1wt_1wt_2wt_2$	12.5	Male sterile Herbicide tolerant	12.5

442. Backcrossing the herbicide susceptible hybrid phenotypes to the wild type (eg. conventional canola) would result in 100 % fertile, non-GM, herbicide susceptible plants. Backcrossing the fertile, RF hemizygous plants with the wild type would result in 50 % of progeny with a fertile, glufosinate ammonium-tolerant phenotype and 50 % fertile, non-GM, herbicide susceptible phenotype. Backcrossing the fertile, RF hemizygous and MS hemizygous with the wild type would result in 50 % of plants with fertile, glufosinate ammonium tolerant phenotypes, 25 % fertile, non-GM, herbicide susceptible phenotype and 25 % sterile and herbicide tolerant. Backcrossing the male sterile, MS hemizygous glufosinate ammonium tolerant plants with the wild type would result in 50 % fertile, non-GM, herbicide susceptible plants and 50 % male sterile and glufosinate ammonium- tolerant plants. Therefore, in the absence of selective pressure (glufosinate ammonium application), the proportion of herbicide tolerant and male sterile phenotypes should decrease with each backcrossed generation.
443. These proportions are what would be expected for random mating but the likelihood of genes occurring and spreading in the field will be influenced by a number of factors, including the level of self-pollination, physical proximity and flowering synchrony.
444. These calculations provide an indication of the proportion of progeny plants that might possess the introduced genes in the absence of any selective advantage.

1.2.3 Transfer of genes between RF x MS hybrids - volunteers

445. Due to seed persistence, volunteers from previous InVigor[®] hybrid crops may emerge in subsequent crops in the field. Because InVigor[®] hybrid seed consists of two genotypes in equal proportions, if allowed to flower and cross-pollinate with each other, 9 different genotypes can result (Table 2). Approximately 86 % of progeny would remain glufosinate ammonium-tolerant, a small proportion of which would also be male sterile (11 %). The remaining 14 % of progeny would not contain any transgenes and would be herbicide susceptible. Mendelian segregation dictates that slight losses in herbicide tolerance would occur in the following backcross generations.

Table 2: Results of the four possible crosses between the 2 hybrid InVigor[®] canola genotypes assuming that each cross provides 25% of the progeny.

Type	Genotype	Genotype (%)	Phenotype	Phenotype (%)
1	w ₁ w ₁ w ₂ w ₂	14.0625	Fertile Herbicide susceptible No transgenes	14.0625
2	w ₁ w ₁ w ₂ RF ₂	28.125	Fertile Herbicide tolerant	75
3	w ₁ w ₁ RF ₂ RF ₂	14.0625		
4	MS ₁ w ₁ w ₂ RF ₂	18.75		
5	MS ₁ w ₁ RF ₂ RF ₂	9.375		
6	MS ₁ MS ₁ w ₂ RF ₂	3.125		
7	MS ₁ MS ₁ RF ₂ RF ₂	1.5625		
8	MS ₁ w ₁ w ₂ w ₂	9.375		
9	MS ₁ MS ₁ w ₂ w ₂	1.5625		

446. In a field situation, between 53 and 88 % of plants are self-pollinated. Self-fertilisation generates the same phenotypes as outcrossing between hybrid phenotypes, although the proportions differ slightly (Table 3). The average proportion of progeny from self-fertilisation of hybrids, assuming that both hybrid genotypes occur in equal proportions, are similar to those in Table 2, with the same proportion of fertile and herbicide tolerant plants, slightly more plants with no transgenes and slightly fewer male sterile and herbicide tolerant plants.

Table 3: Results of self-fertilisation of the two hybrid genotypes assuming that each cross is present in equal proportions.

Type	Genotype	Genotype (%)	Phenotype	Phenotype (%)
1	$w_1w_1w_2w_2$	15.625	Fertile Herbicide susceptible No transgenes	15.625
2	$w_1w_1w_2RF_2$	31.25	Fertile Herbicide tolerant	75
3	$w_1w_1RF_2RF_2$	15.625		
4	$MS_1w_1w_2RF_2$	12.5		
5	$MS_1w_1RF_2RF_2$	6.25		
6	$MS_1MS_1w_2RF_2$	6.25		
7	$MS_1MS_1RF_2RF_2$	3.125		
8	$MS_1w_1w_2w_2$	6.25	Male Sterile Herbicide tolerant	9.375
9	$MS_1MS_1w_2w_2$	3.125		

447. Under commercial situations, no hybrid system can produce 100 % hybrid seed. There is always a small proportion of MS and RF lines that are present in the hybrid seed. However, the proportion of these plants that is present in seed is so small that they can be ignored when considering these percentages.

1.2.4 Outcrossing with herbicide tolerant canola

448. Development of tolerance to multiple herbicides (gene stacking) in canola volunteers has been observed in commercial situations in Canada (Downey 1999a; Hall et al. 2000; Beckie et al. 2001). Five herbicide tolerant types of canola have been commercialised in Canada – glufosinate ammonium (GM), glyphosate (GM), bromoxynil (GM), imidazolinone/ALS inhibitors (non-GM) and triazine (non-GM). In 1998, a field of canola was identified as having volunteers with multiple tolerances to glyphosate and/or glufosinate ammonium and/or imidazolinones (Hall et al. 2000). In 1999 a further 11 fields in Canada were confirmed as containing multiple herbicide tolerant volunteers (Beckie et al. 2001).
449. In Canada, the frequency of gene stacking between adjoining glyphosate and glufosinate ammonium-tolerant crops was greatest on the field edge (closest to neighbouring GM crop) at approximately 1%, but within the crop was 0.2 % or less for distances between 50m and 800m, from the edge (Beckie et al. 2001). The maximum distance at which gene flow was detected was 800m. Similar levels of gene flow between glyphosate and glufosinate ammonium-tolerant GM canola crops were recorded in Canada by Downey (1999a) and with glufosinate ammonium-tolerant

and/or glyphosate tolerant GM canola in the U.K. (Scheffler et al. 1993; Simpson et al. 1999; Ingram 2000). The presence of stacked herbicide tolerance genes in the seed that is sown may, in some instances, influence these measurements as recent reports from Canada indicate that some certified seedlots have contamination levels exceeding the maximum 0.25 % standard (Lodish et al. 2000; Downey & Beckie 2002). Further detail is provided below in the section on “Seed Production”.

450. No instances of gene stacking have been recorded in the United States, possibly due to the short period and limited number of regions in which GM herbicide tolerant canola has been commercially grown (Orson 2002). However, canola plants tolerant to glyphosate, glufosinate ammonium and imidazolinones have occurred in field experiments over two years (Orson 2002). Gene stacking has been experimentally demonstrated in France (Champolivier et al. 1999). Canola volunteers tolerant to two herbicides were detected in a series of experiments in France, where three herbicide tolerant canola varieties were sown in adjacent fields at three sites.
451. There are two conventionally bred herbicide-tolerant canola varieties currently being grown throughout Australia – triazine tolerant and imidazolinone-tolerant.

Table 4: Area planted to conventionally bred herbicide susceptible and herbicide tolerant (Clearfield[®] and triazine-tolerant ‘TT’) canola varieties in 2002 (‘000 ha) in each state. Values in parentheses are percentage of area sown. Figures are a guide only*.

	NSW	VIC	SA	WA	TOTAL
Susceptible	120 (30)	48 (20)	13 (10)	7.2 (2)	188.2 (17)
Clearfield [®]	40 (10)	48 (20)	26 (20)	10.8 (3)	124.8 (11)
TT	240 (60)	144 (60)	91 (70)	342 (95)	817 (72)
Total	400 (35)	240 (21)	130 (12)	360 (32)	1130

* Information provided by Canola Association of Australia (monthly crop forecast data), R. Wilson and K. Morthorpe (Pioneer Hi-Bred) and J. Kudnig (Dovuro).

452. A significant proportion of the canola crop in Australia is triazine tolerant, with estimates of between 55% (Norton, 2003) and 70% (Table 4). Triazine tolerant canola represents up to 95% of canola production in Western Australia (Table 4, Norton 2003).
453. Triazine tolerant (‘TT’) canola has been selected to be tolerant to triazine herbicides (Group C) with the resistance originating from a cytoplasmic mutation. The gene conferring resistance is inherited maternally and, therefore, cannot be spread to neighbouring paddocks by pollen movement. The triazine resistance mechanism also imparts a physiological penalty to the plant resulting in reduced fitness (Powles et al. 1997). Triazine tolerant canola continues to have a yield disadvantage of 10-15 % and about 3-5 % lower oil content than conventional varieties but is accepted by farmers because it allows canola to be grown where Brassicaceous weeds are a problem (Colton & Potter 1999).
454. Imidazolinone tolerant (Clearfield[®], ‘IT’ or ‘Imi’) canola is resistant to imidazolinone herbicides (Group B). The tolerance is produced by a mutation which confers tolerance to inhibitors of the enzyme acetolactate synthase (ALS) in two nuclear genes and as a result the resistance genes can be carried in pollen. There are a number of herbicides that are ALS inhibitors. Clearfield[®] cultivars released for commercial

- production are homozygous for both genes. However, since the genes do not confer an equal level of resistance, hybridisation between non-imidazolinone tolerant or hemizygous imidazolinone tolerant plants will result in progeny with levels of imidazolinone tolerance depending on the gene(s) present and their copy number (homozygous or hemizygous). Clearfield[®] was introduced into Australia in 2000 and represents between 5 and 10% of production (Table 4, Norton 2003).
455. Hybridisation between existing conventional herbicide-tolerant canola varieties and glufosinate ammonium-tolerant GM canola would result in accumulation or 'stacking' of genes for tolerance to up to three different herbicide groups within the same plant.
 456. The Regulator is also considering an application for commercial release of glyphosate tolerant GM canola (Monsanto Australia Ltd – DIR 020/2001). Hybridisation between glyphosate tolerant GM canola (Roundup Ready[®] canola) and InVigor[®] canola or conventional herbicide tolerant varieties would also result in accumulation or 'stacking' of genes for tolerance to multiple herbicides within the same plant. Senior et al. (2002) found that stacking together glufosinate ammonium and glyphosate tolerance traits into both winter and spring lines of canola did not alter its susceptibility to other, unrelated herbicides, and no gene silencing was observed.
 457. As discussed previously, some pollen flow between crops is inevitable. While not a risk to human health and safety or the environment, post-emergence control of gene stacked volunteers in a subsequent canola crop might affect the choice of herbicides for weed control operations on-farm.
 458. In Canada, five herbicide tolerant canola types have been commercialised, including glyphosate tolerant canola. Multiple herbicide tolerant volunteers are generally managed by the addition of a low rate of 2,4-D to the pre-sowing application of glyphosate, while those volunteers emerging with the crop are controlled by post-emergence herbicides (Orson 2002). Phenoxy herbicides have to be used post-emergence in cereals where volunteers contain the gene(s) for imidazolinone tolerance (Clearfield[®]) that results in tolerance to ALS inhibitor herbicides, such as sulfonylureas, which are commonly used for weed control in wheat. In Canada where canola is grown no more than once in four years, surveys have shown that the numbers surviving from the previous crop are less than half of one plant per square metre (Legere et al. 2001; Simard et al. 2002).
 459. Management of multiple herbicide tolerant canola that might result from hybridisation between herbicide tolerant varieties (GM and/or non-GM) can be achieved by the application of the already established principles and practices for minimising the development of herbicide resistance in any agricultural weed: informed selection and rotation of herbicides and crops; attention to the control of volunteers; maintenance of hygiene in seeding; harvesting and transport operations; and implementation of good agronomic practices.
 460. Bayer's InVigor[®] Canola Crop Management Plan recommends that farmers should anticipate multiple herbicide tolerance in order to effectively control canola volunteers. To minimise the potential for gene flow they recommend that growers:
 - slash, cultivate or harvest and process approximately 5 m of any adjacent non-GM canola crop as part of the GM crop;
 - notify adjoining land holders if a GM crop is grown along a boundary adjacent to a neighbouring canola crop;
 - use clean machinery and trucks to reduce spread of GM seed;

- scout fields to identify herbicide tolerant canola in succeeding crops and control them using herbicides, grazing or cultivation;
 - use proper rotations to allow removal of volunteers; and
 - keep accurate field records.
461. The recommendations in the CMP for the implementation of a 5m buffer also relate to addressing possible market requirements or thresholds regarding the adventitious presence of GM canola and not human health and safety or environment issues.
462. Implementation of a 5m buffer between adjacent GM and non-GM canola fields would not preclude gene flow between the two crops.
463. It is well established that the rate of cross-pollination between canola decreases significantly over the first 5-10 metres and the work of Rieger et al. (2002) supports the conclusion that the amount of gene flow between commercial canola fields would be below 1% on a paddock basis (Rieger et al. 2002). Recent work by Reboud (2002) demonstrated that the level of cross pollination between adjacent canola crops was the same if they were separated by a clear gap of 3-4 m or 1 m of the adjoining edge of the crop removed after flowering.
464. Bayer also recommends that where a herbicide-tolerant crop will be grown along a boundary fence line that is adjacent to a neighbouring canola crop, that the farmer notify the adjoining land owner. Arrangements between individual growers regarding the establishment of any such buffers are obviously outside the scope of this assessment.
465. Glufosinate ammonium is a group N herbicide. Each herbicide group has a different mode of action and glufosinate ammonium is the only group N herbicide registered in Australia.
466. Bayer made a parallel application to the APVMA for registration of glufosinate ammonium for use on InVigor[®] canola under the trade name Liberty[®]. The APVMA has registered Liberty[®] for use only InVigor[®] canola crops, not for weed control in other crops (APVMA 2003). Glufosinate ammonium is not registered for use in any other broad-acre cropping in Australia.
467. The management options available to control GM glufosinate ammonium tolerant canola are no different to those already available to control conventional and non-GM herbicide tolerant volunteer canola. The addition of glufosinate ammonium-tolerant canola to the cropping system will not require a change in the type of herbicides currently used to control canola volunteers.
468. The potential impact of multiple herbicide tolerant canola on natural habitats is low. As previously stated, canola is a plant of disturbed habitats and plants with multiple herbicide tolerance will be no more weedy or invasive than single herbicide tolerant or non-herbicide tolerant canola types. As glufosinate ammonium is not used in undisturbed natural habitats in Australia (Dignam 2001), canola plants tolerant to glufosinate ammonium would have no selective advantage in these environments.
469. If stacking of a glufosinate ammonium tolerance gene into already herbicide tolerant canola occurred, it would not alter the herbicide management options available for control. Furthermore, appropriate volunteer management, proper crop rotation and herbicide management practices should limit the possibility of multiple herbicide tolerance occurring as a result of cross-pollination (Rieger et al. 2001; Downey 1999a; Salisbury 2002).

MALE STERILE AND FERTILITY RESTORER LINES

470. The fertility restorer gene would have no impact on a plant's phenotype apart from restoring male fertility for a portion of the progeny of a plant with the male sterile gene.

SEED PRODUCTION

471. Bayer has indicated that the creation of the MS and RF plants and their subsequent crossing, resulting in the InVigor[®] hybrid seed, which is distributed to growers, occurs as part of a process which is consistent with the industry standards for the production of certified canola seed. As such, strict quality assurance protocols are followed to ensure that crops are isolated from other canola crops by a minimum distance of 400 m, thereby minimising the level of contamination by surrounding canola crops. This also limits the potential for gene transfer to occur from the MS and RF lines to surrounding canola crops.
472. A recent Canadian study has reported levels of contamination in certified seed lots of canola which exceed their industry standards (Downey & Beckie 2002). Seventy samples from 14 varieties of herbicide susceptible varieties were screened for the presence of genetically modified herbicide tolerance genes, including glufosinate tolerance, using selective herbicide tests. In 10 of the 14 varieties tested, the average level of contamination was below the 0.25 % maximum contamination standard set for certified seed by the Association of Official Seed Certifying Agencies. In the 4 varieties where the 0.25 % standard was exceeded (0.28-0.81 %), contamination was attributed to mixing during seeding, harvesting or cleaning operations or to variety development, rather than to outcrossing during seed production.
473. Glufosinate ammonium seedlings were present in 20 % of samples, 50 % contained glyphosate tolerant-tolerant seedlings and 15 % had seedlings that were tolerant to both herbicides.
474. Another recent Canadian survey of 15 conventional, glufosinate tolerant (Liberty Link[®]) and Clearfield[®] canola varieties also found levels of contamination that exceeded the 0.25% standard (Friesen et al. 2003). Roundup Ready[®] varieties were not detected. Samples were tested for resistance to glyphosate, glufosinate ammonium (Liberty[®]), thifensulfuron (a herbicide to which Clearfield[®] varieties are tolerant) and mixtures of these herbicides. The 33 certified seedlot samples collected represented 27 unique certified seedlots. Of the 33 seedlots sampled, only 1 seedlot had no detectable contamination. Of the 27 unique certified seedlots, 14 had contamination levels above 0.25 % with 9 contaminated with the glyphosate tolerance trait. Three seedlots had glyphosate tolerance contamination levels in excess of 2 %. The remaining 5 contaminated seedlots were contaminated with levels above 0.25% of glufosinate ammonium-tolerance trait (20 seedlots were glufosinate ammonium-susceptible). Interestingly, six of the seven glufosinate ammonium-tolerant seedlots had lower levels of individual tolerance to both glyphosate and glufosinate ammonium compared to the level of individuals tolerant to glyphosate, indicating that the ostensibly glufosinate tolerant seedlots may have been contaminated with susceptible varieties. There was very little contamination of seedlots with the Clearfield[®] resistance trait.
475. These results clearly demonstrate that the introduction of herbicide tolerance traits, whether GM or conventionally derived, has provided an extremely sensitive method of detecting contamination in seed stocks which is not possible with non-herbicide tolerant varieties. The example also suggests that in the absence of such sensitive

discriminatory characters the levels of contamination of canola seed lots might be underestimated. Although instances of significant seedlot contamination were attributable to causes other than gene flow, these results from Canada may have implications for the standards for the production of certified canola seed (both GM and non-GM) in Australia and elsewhere.

Section 1.3 Conclusions regarding gene transfer to other canola plants

476. Canola is mainly self-pollinating but outcrossing between adjacent plants does occur at significant rates (approximately 30 %). The highest rates of outcrossing are between adjacent plants (less than 5m), and the rate decreases significantly at distances of over 5-10m. Under Australian conditions, outcrossing rates between commercial canola crops have been shown to be well below 0.2 % in the majority of cases. Outcrossing can be detected at greater distances (detected up to 2.6km under Australian conditions), but at extremely low levels.
477. In a commercial situation low levels of outcrossing between canola varieties is inevitable. However the transfer of the bar herbicide tolerance gene from the GM canola to other canola will not confer a competitive or ecological advantage to these plants in the absence of glufosinate ammonium, and the hazards are the same as for the GM canola lines.
478. In broadacre agriculture in Australia glufosinate ammonium is only registered as Liberty[®] for use on InVigor[®] hybrid canola. Glufosinate ammonium tolerant canola volunteers can be controlled by the same herbicide and cultural practices currently used to control conventional and non-GM herbicide tolerant canola volunteers. These plants are no more likely to become weeds of cropped areas than conventional canola plants. Liberty[®] would not be used for the control of canola volunteers.
479. In situations where canola varieties resistant to different herbicides are grown in proximity, the occurrence of multiple herbicide resistant canola volunteers resulting from outcrossing will be inevitable. However, multiple herbicide resistant plants can be readily controlled by alternative herbicides and cultural practices. Furthermore, the development of volunteers with resistance to a number of herbicides can be minimised by good management practices both on and off farm.
480. Outside broad acre cropping the use of glufosinate ammonium for weed control in Australia is limited to areas where grapevines, fruit trees and vegetables are grown or in areas associated with agricultural non-crop areas, commercial and industrial areas and rights-of-way. There is a wide range of alternative herbicides and cultural practices available to control glufosinate ammonium-tolerant canola that may appear as a weed in these situations. Furthermore, glufosinate ammonium-tolerant plants have no selective advantage in the absence of application of glufosinate ammonium and can be controlled by the same herbicide and cultural practices currently used to control conventional canola and the risks are the same as those applying to the intentionally cultivated GMO.
481. The likelihood of gene transfer from the glufosinate ammonium tolerant GM canola to other canola is high, but it will not result in adverse impacts to human health and safety or the environment. The risk associated with gene transfer to other canola is therefore concluded to be negligible and no management conditions are required for this release.

SECTION 2 TRANSFER OF INTRODUCED GENES TO OTHER PLANTS

482. This section will focus on the likelihood of gene flow (transfer) and introgression from InVigor[®] hybrid canola to related Brassicaceae species and make conclusions about the consequences of these risks for the environment.

Section 2.1 Nature of the gene transfer hazard

483. Transfer of the introduced genes into other plant species, in particular to weedy relatives, might produce weeds that are more competitive or invasive and have adverse effects on biodiversity. The potential hazards specific to the transferred gene sequences are as follows:

- Herbicide tolerance gene (*bar / pat* gene)
Plants could become tolerant to glufosinate ammonium. This would have an impact in situations where glufosinate ammonium is used.
- Male sterility gene (*barnase* gene)
Male sterile plants are unable to produce pollen and can only reproduce by receiving foreign pollen. Transfer of the *barnase* gene from InVigor[®] hybrid canola plants to other species would result in male sterility in a proportion of interspecific hybrids.
- Fertility restorer gene (*barstar* gene)
Plants carrying the fertility restorer gene would be fully fertile and have no impact on other plants.
- Antibiotic resistance gene (*nptII*)
Antibiotic resistance gene (*nptII*) is present only in Topas19/2, RF1, RF2, MS1 and not in RF3, MS8 which are proposed for commercialisation and has no impact on other plants. No relevant phenotypic effect with gene transfer to plants
- Promoters and other regulatory sequences

If gene transfer did occur, there could be unintended or unexpected effects if the introduced regulatory sequences altered the expression of endogenous plant genes. Some regulatory sequences introduced into the GM canola lines are derived from plant pathogens (*Agrobacterium tumefaciens*, Cauliflower Mosaic Virus).

Section 2.2 Likelihood of the gene transfer hazard occurring

484. For transgenes to flow from InVigor[®] canola to other plants and persist in the recipient plants, the first step is the production of spontaneous interspecific hybrids. The proportions of herbicide tolerant and susceptible progeny expected to be produced from crosses with InVigor[®] canola, the male sterile (MS) or fertility restorer (RF) lines and related brassicaceous species, are the same as for a cross with *B. napus* (refer to Section 1 of this Appendix). The *bar* and *barnase* (MS) or *barstar* (RF) genes are completely linked and are passed from one plant to another as a single locus.

485. A number of factors influence the likelihood of gene flow occurring. Pre-fertilisation considerations include physical proximity and pollen movement, synchrony of flowering, breeding system and floral characteristics and competitiveness of interspecific pollen. Post-fertilisation considerations include sexual compatibility, hybrid viability and fertility, viability and fertility of progeny through several generations of backcrossing and successful introgression (incorporation of the modified

genes into the genome of the weedy species). For successful gene transfer to occur, all pre- and post-fertilisation requirements must be met. Failure to meet any one requirement will mean that gene transfer and introgression cannot occur.

486. Following the initial hybridisation event, efficient gene flow from crop to weedy species requires the production of successive generations that retain the modification in a functional way (Chevre et al. 2001). Persistence of the transgenes then depends on either stable introgression of transgenes within natural populations or the stabilisation of the hybrid form leading to the creation of a new weed (Chevre et al. 2001). Both of these possibilities depends on the fertility, genomic structure, vigour of the progeny, sexual compatibility of progeny with the wild type and the transmission of InVigor[®] canola genes within successive generations.
487. Interspecific hybrids, which can result from an initial cross between canola and a related species, may have low fertility or reduced vigour and consequently only a small chance of persisting. Repeated backcrossing of the hybrid with wild plants can lead to gradual introgression of the gene in question into the wild population.
488. The most likely possibility of gene transfer to other plant species would be transfer to other Brassica species or sexually related Brassicaceae species, although this is far less likely than transfer to other canola plants. Transfer to unrelated plant species can be considered highly improbable, and no evidence has been identified for any horizontal gene transfer mechanism by which this could occur.
489. Salisbury (2002) has summarised the potential for gene flow between canola (*B. napus*) and Brassicaceae species found in Australia (Table 2).

2.2.1 Introgression of genes of *Brassica napus* vegetables and forage rape

490. Gene flow is possible from *B. napus* canola to *B. napus* forage rape and vegetables such as swedes, rutabaga and Siberian kale (Salisbury 2002). However, since *B. napus* vegetables are generally harvested before flowering and are not recognised as weeds in agricultural or natural habitats, there is limited potential for the acquisition of herbicide resistance genes unless being used as a seed production crop. Seed production crops are isolated from other *B. napus* crops to prevent outcrossing. Flowering synchrony is also required for pollen transfer to occur. Forage rape crops rarely flower and are usually consumed by foraging animals before seed development.

2.2.2 Introgression of genes into other *Brassica* species

491. Field hybrids and introgression of foreign genes has been demonstrated for *B. rapa* and *B. juncea*. *Brassica napus* (AACC) shares a common set of chromosomes with *B. rapa* (AA), *B. juncea* (AABB) and *B. oleracea* (CC).

Table 2. Potential gene flow between canola (*B. napus*) & Australian Brassicaceae species (Salisbury 2002)

Category	I	II	III	IV	V	VI
Tribe	<i>Brassicaceae</i>	<i>Brassicaceae</i>	<i>Brassicaceae</i>	<i>Brassicaceae</i>	<i>Brassicaceae</i>	Other
Glasshouse 'rescued' hybrids	Yes	Yes	Yes	Yes	No	No
Glasshouse hand hybrids	Yes	Yes	Yes	No	No	No
Field hybrids	Yes	Yes ²	Not reported	Not reported		
Gene introgression	Yes/Likely ¹	Not reported				
Weeds	<i>Brassica rapa</i> <i>Brassica juncea</i> ¹	<i>Raphanus raphanistrum</i> <i>Hirschfeldia incana</i> <i>Sinapis arvensis</i>	<i>Brassica fruticulosa</i> <i>Brassica nigra</i> <i>Brassica tournefortii</i> <i>Diplotaxis muralis</i> <i>Diplotaxis tenuifolia</i> <i>Rapistrum rugosum</i>	<i>Brassica oxyrrhina</i> <i>Diplotaxis tenuisiliqua</i>	<i>Conringia orientalis</i> <i>Carrichtera annua</i> <i>Cakile maritima</i>	<i>Capsella bursapastoris</i> <i>Cardaria draba</i> <i>Lepidium sp.</i> <i>Myagrimum perfoliatum</i> <i>Sisymbrium orientale</i> <i>Sisymbrium irio</i> <i>Sisymbrium erysimoides</i> <i>Sisymbrium officinale</i>
Condiment, fodder & vegetable species	Forage <i>B. napus</i> ¹ <i>B. napus</i> vegetables ¹ <i>B. rapa</i> vegetables ¹ Condiment <i>B. juncea</i> ¹		<i>Brassica alboglabra</i> ³ <i>Brassica chinensis</i> ⁴ <i>Brassica nigra</i> <i>Brassica oleracea</i> <i>Brassica pekinensis</i> ⁴ <i>Raphanus sativus</i> <i>Sinapis alba</i>			

→ DECREASING SEXUAL COMPATIBILITY →

¹ Considered likely to happen over a period of time if the species are in physical proximity and have flowering synchrony.² Frequency of interspecific hybrids approx. 10^{-4} to 10^{-8} . Likelihood of subsequent introgression or formation of fertile amphidiploids significantly less again.³ This species is sometimes considered to be a subspecies of *B. oleracea*.⁴ These species have sometimes been considered to be subspecies of *B. rapa*.

Table 2 (cont.). Potential gene flow between canola (*B. napus*) & Australian *Brassicaceae* species.

Category	I	II	III	IV	V	VI
Tribe	<i>Brassicaceae</i>	<i>Brassicaceae</i>	<i>Brassicaceae</i>	<i>Brassicaceae</i>	<i>Brassicaceae</i>	Other
Glasshouse 'rescued' hybrids	Yes	Yes	Yes	Yes	No	No
Glasshouse hand hybrids	Yes	Yes	Yes	No	No	No
Field hybrids	Yes	Yes	Not reported	Not reported		
Gene introgression	Yes/Likely#	Not reported*				
<i>Native species</i>						<i>Arabidella</i> (6 sp.) <i>Balbaretinia</i> (1 sp.) <i>Barbarea</i> (2 sp.) <i>Blennodia</i> (25 sp.) <i>Cardamine</i> (5 sp.) <i>Carinavalva</i> (1 sp.) <i>Cheesemania</i> (1 sp.) <i>Cuphonotus</i> (2 sp.) <i>Geococcus</i> (1 sp.) <i>Harmsiodoxa</i> (3 sp.) <i>Irenepharsus</i> (3sp.) <i>Lepidium</i> (35 sp.) <i>Menkea</i> (6 sp.) <i>Microlepidium</i> (2 sp.) <i>Pachymitus</i> (1 sp.) <i>Phlegmatospermum</i> (4 sp.) <i>Rorippa</i> (4 sp.) <i>Scambopus</i> (1 sp.) <i>Stenopetalum</i> (9 sp.)

→ DECREASING SEXUAL COMPATIBILITY →

Brassica rapa

492. *Brassica rapa* (= *B. campestris*) is found throughout Queensland, New South Wales, Victoria and South Australia and sometimes occurs as a weed of disturbed and cultivated land, however it is not a weed of undisturbed natural areas (Auld & Medd 1987; Groves et al. 2000). *B. rapa* is reported as a minor weed in New South Wales, Victoria, Queensland, South Australia and Western Australia (Hyde-Wyatt & Morris 1989; Holm et al. 1997; Groves et al. 2000; Groves et al. 2002) however other reports indicate that it is not a widespread agricultural weed (Hussey et al. 1997; Salisbury 2002).
493. *Brassica rapa* is considered a major weed of disturbed environments throughout Tasmania and occurs in arable crops, along roadsides and in waste areas in that state (Anon. 2002, Groves et al. 2002). The incidence of *B. rapa* is particularly concentrated in a few specific locations in Tasmania, especially the Coal River valley, around the mouth of the Derwent River, on heavy red soils around Scottsdale, and on the north-west coast between Deloraine and Ulverstone (S. Smith pers. comm.).
494. Therefore, the possibility that the genes encoding tolerance to glufosinate ammonium will be transferred to hybrids and introgress into weedy *B. rapa* populations is most likely to occur in Tasmania.
495. Hybrids between *B. napus* and *B. rapa* have not been reported to date in Australia, except putatively in plant breeders nurseries (Salisbury 2002).
496. Several subspecies of *B. rapa* are recognised, including *B. rapa* ssp. *sylvestris*, *B. rapa* ssp. *rapa* and *B. rapa* ssp. *oleifera*. *B. rapa* ssp. *oleifera* is cultivated in North America and Europe as an oilseed or forage crop. It was cultivated as an oilseed in Australia but has since been replaced by *B. napus* (Salisbury 2002), but it is still grown as a forage crop ('forage rape') in Australia, sometimes as a mixture with *B. napus* (information supplied by Bayer). *B. rapa* ssp. *rapa* is the vegetable turnip. The weedy form of *B. rapa* is usually considered to be *B. rapa* ssp. *sylvestris*. (In the following discussion of *B. rapa* as a weed it is assumed that it is *B. rapa* ssp. *sylvestris* unless otherwise indicated)
497. *Brassica rapa* has seed dormancy and seed longevity and seeds may persist in the soil for many years (Canadian Food Inspection Agency 1999). *B. rapa* is self-incompatible and is an obligate outcrosser (Jorgensen & Andersen 1994, Salisbury 2002).
498. There have been many reports of hybrids being formed between *B. rapa* and *B. napus*, both in field and experimental situations (Bing et al. 1996; Jorgensen et al. 1996; Brown & Brown 1996; Halfhill et al. 2002; Warwick et al. 2003). Gene flow can occur in either direction but where it occurs in a crop with *B. napus* as the female, most of the hybrid seed would be harvested and removed along with the canola. In general, more hybrids are found with *B. rapa* as the female as *B. rapa* is self-incompatible and an obligate outcrosser (Jorgensen & Andersen 1994; Salisbury 2002). In addition, pollen from both *B. rapa* and *B. napus* has equal fitness when applied to *B. rapa* stigmas and so either species is equally likely to fertilise *B. rapa* (Hauser et al. 1998b).
499. The reported rates of outcrossing between *B. rapa* and *B. napus* vary significantly, and the rate depends on the situation (Eastham & Sweet 2002). The genotypes of both *B. napus* and *B. rapa* also affect the rate of hybridisation (Jørgensen et al. 1998; Norris & Sweet 2003), and some genotype combinations may be incompatible (Norris & Sweet, 2003). Field studies have tended to focus on identifying hybrids from progeny (seeds)

- of *B. rapa* plants (ie *B. rapa* as mother) as evidenced by transfer of a marker gene, especially herbicide tolerance to *B. rapa*.
500. Low levels of hybridisation have been observed in a number of studies. A study by Scott and Wilkinson (1998) found low levels of hybrids (0.4 - 1.5 %) in natural populations of *B. rapa* growing in close proximity (1 - 5 m) to large fields of canola with only 2 % of hybrid seedlings surviving. Investigation of hybridisation from GM glufosinate ammonium tolerant canola line HCN92 (equivalent to Topas 19/2) to *B. rapa* under field conditions found a hybridisation frequency of 3.3% (data supplied by Bayer).
 501. Intermediate rates of hybrid formation seem to be achieved when there are mixed population of *B. rapa* and *B. napus*, either in experimentally plantings or natural populations. In mixed stand of *B. napus* and *B. rapa* Jorgensen et al. (1996) found hybridisation rates of 13% with *B. rapa* as the female and 9% on *B. napus* as the female. Similarly Kvaloy (2001) reported hybridisation rates of between 2 and 6% hybrids from *B. napus* in experimental mixed stands with *B. rapa* in Norway.
 502. The highest rates of hybridisation have been observed for single *B. rapa* plants growing in fields of *B. napus* with up to 93% of F1 progeny seeds from *B. rapa* being hybrids (Jorgensen et al. 1996). *B. rapa* is an obligate outcrosser and in such situations there is very great pollen competition from surrounding *B. napus*.
 503. When *B. rapa* is separated from *B. napus* the rate of hybridisation is low. Norris and Sweet screened for hybrid seeds from a plot of commercial *B. rapa* ssp. *oleifera* ('turnip rape', 0.12 ha) grown adjacent to a field of GM glufosinate ammonium tolerant canola (0.8 ha). They found the average rate of hybridisation was 0.25% at 1 m, 0.008% at 41 m and zero at 51 m (Norris & Sweet 2003). Norris & Sweet (2003) did not detect any inter-specific hybrids in a UK field of *B. rapa* ('stubble turnips') 400 metres from a trial plot of glufosinate ammonium tolerant canola by herbicide spot testing, however they only tested 35 plants. Survey studies of past trial sites in Tasmania have failed to detect any gene transfer from GM canola to *B. rapa* (Rieger 2002; Agronico 2002).
 504. In field experiments conducted in Canada with individual *B. rapa* plants positioned within or adjacent to (0.5m from the edge) plots of GM glyphosate tolerant *B. napus* hybridisation rates were between 3.4% - 8.3% (*B. rapa* as mother), with the lowest rates at the margin (2003). All F1 hybrids were morphologically similar to *B. rapa* and had reduced pollen viability (average 54%). However a large proportion of the hybrids were self-fertile.
 505. There may also be a genotype interaction between *B. napus* and *B. rapa* which may affect the rate and success of hybridisation. A study with *B. rapa* bait plants adjacent to GM glufosinate ammonium or glyphosate tolerant canola fields demonstrated herbicide tolerant hybrid formation, but some plants did not set any seed which was attributed to genetic incompatibility between some *B. rapa* and canola varieties (Norris & Sweet 2003). Hauser et al. (2001) also reported that hybrid and backcross offspring were produced mainly by a few of the *B. rapa* plants, indicating that the degree of hybridisation and backcrossing may dependent on the *B. rapa* genotype.
 506. Norris and Sweet (2003) have demonstrated extensive hybridisation and backcrossing in both directions between *B. napus* and weedy *B. rapa* at a site sown to three different non-GM canola cultivars between 1988 and 1996. The site was sown to GM glufosinate ammonium tolerant canola in 1998. Hybrids were identified by

- morphology, flow cytometry and AFLP analysis, both in plants collected during the 1998 season and in soil core seed samples from the site. F1 progeny from 5 individual *B. rapa* plants within the GM glufosinate ammonium tolerant canola field were tested for glufosinate ammonium tolerance and on average 11.3% were determined to be hybrids, although two plants produced no hybrids (Norris & Sweet 2003).
507. Hybrids identified in the field were fertile but their anthers were reduced in size or absent in some cases, and pollen and seed production was low compared to either *B. rapa* or *B. napus*. Seed pods were often empty or contained very few seeds and many seeds were aborted, shrivelled or malformed, and seeds often germinated in the pod (Norris & Sweet 2003). Hauser and Ostergard (1999) also reported germination of hybrid seeds within pods.
 508. In Canada, Warwick et al (2003) found an average hybridisation rate in a commercial field of glyphosate tolerant canola to *B. rapa* was 13.6%, ranging from 0 – 53.3% per plant. They also detected hybridisation from volunteer canola to *B. rapa* in a corn field sown to canola the previous year, with one hybrid in 4259 seeds sampled (0.023%). The *B. rapa* were at the field margin and separated from *B. napus* plants by 0.5 – 5m.
 509. Hybrids from transgenic glufosinate ammonium tolerant *B. napus* and wild *B. rapa* crosses under glasshouse conditions resulted in herbicide tolerance being transmitted to the third backcross generation (BC3) at an average frequency of 50 %, as would be expected for a dominant Mendelian trait (Snow & Jorgensen 1999). Pollen fertility (88-95 %) and seed set of the BC3 was not significantly different to that of non-transgenic *B. rapa* plants raised in the same glasshouse. These results suggest that transgenic herbicide tolerance is capable of introgressing and persisting in *B. rapa* populations, even in the absence of selection due to herbicide applications.
 510. Halfhill et al. (2002) demonstrated hybridisation between Bt GM *B. napus* and *B. rapa*, with *B. napus* as pollen donor, both in glasshouse and field experiments, and introgression of the transgene up to the second backcross generation with *B. rapa* in hand pollination experiments.
 511. A number of studies have demonstrated that *B. rapa* x *B. napus* hybrids have reduced fertility, seed set and fitness (Scott & Wilkinson 1999; Jorgensen & Andersen 1994; Hauser & Ostergard 1999; Norris & Sweet 2003). However other studies have demonstrated that hybrids may have increased reproductive fitness relative to the parents (Hauser et al. 1998b).
 512. Recent studies have provided evidence that the fitness of hybrids between *B. napus* and *B. rapa* may be strongly frequency dependent (Hauser et al. 1998a; Hauser et al. 1998b; Pertl et al. 2002; Hauser et al. 2003).
 513. Pertl et al. (2002) measured the effect of planting density and different proportions of *B. napus*, *B. rapa* and their F₁ hybrids on their fitness when pollinating *B. rapa* plants. They found that flowering periods of the two species and the F₁ hybrids overlapped extensively and that plants at low density (16m²) produced more flowers and flowered later than at high planting density (100m²). F₁ plants produced many more open flowers than their parents especially when growing at low density and produced more seeds/plant than *B. rapa* or *B. napus*. Thus female fitness of F₁ hybrids was much higher than that of the parental types and seed set was found to be independent of the relative proportions of *B. rapa*, *B. napus* and F₁ hybrids in the field. Hauser et al. (2003) also demonstrated that F₁ hybrids and backcross progeny had increased female fitness as measured by seed production, and that this was strongly influenced by the

- frequency of hybrid and parental plants, with F₁ hybrids producing many more seeds in mixtures than in pure stands.
514. In contrast male fitness was found to be much lower in the F₁ hybrids. Although the number of pollen grains produced per flower was similar among *B. rapa*, *B. napus* and the F₁ hybrids, pollen viability was much lower in the F₁ hybrids than the parents and declined slightly over the season. Furthermore both F₁ hybrids and *B. napus* almost only sired offspring when at high frequencies themselves. The number of F₁ and backcross offspring was also less than expected at low planting densities.
 515. The implication of these results is that although female fitness may be much higher in F₁ hybrids there will be little opportunity for this to be expressed in an agricultural context because these hybrids (*B. rapa* male; *B. napus* female) are likely to be more abundant in-crop and can be controlled as part of the normal weed control process before and during cropping. Furthermore, the fitness of F₁ hybrids where *B. napus* is the pollen donor and *B. rapa* is the female is low because *B. napus* is successful at pollinating *B. rapa* females only when the relative proportion of *B. napus* is much higher than *B. rapa* or F₁ hybrids and at low planting densities. Finally, although hybrids are likely to be found around the edges of fields, their overall fitness is predicted to be lower than *B. rapa* weeds or *B. napus* volunteers.
 516. Norris and Sweet (2003) have suggested that weed management practices may affect the likelihood of hybridisation and of backcrossing between *B. napus* and *B. rapa*. They postulate that in situations where weed management is effective, individual *B. rapa* plants might be isolated within a canola field, increasing the likelihood of hybrids resulting from *B. rapa* being pollinated by canola, but that if weed management is poor and the frequency of *B. rapa* plants is higher there may be less hybrid formation. Backcrossing is more likely if *B. rapa* is abundant as a result of poor weed management (Norris & Sweet 2003)
 517. Following hybridisation, backcrossing to wild populations is required for introgression of transgenes to occur. Backcrossing of *B. napus* x *B. rapa* hybrids has been demonstrated both experimentally and in the field (Hauser et al. 1998a; Hauser et al. 1998b; Snow et al. 1999; Hansen et al. 2001; Norris & Sweet 2003; Hauser et al. 2003).
 518. For example, in Denmark, weedy *B. napus* and *B. rapa* plants were collected from a field which had produced organic crops in the previous 10 years (Hansen et al. 2001). No canola had been grown since the site had been converted to organic farming. Of the 102 Brassica plants screened with 24 species-specific AFLP markers, 44 plants appeared to be introgressed beyond the F₁ generation.
 519. In the UK, Norris and Sweet (2003) found evidence of significant hybridisation and backcrossing between *B. napus* and *B. rapa* coexisting in a commercial field (as described above), and AFLP analyses indicated that introgression was occurring.
 520. Since *B. rapa* and *B. napus* share the A-chromosomes in common, it has been suggested that transgenes integrated on a C-chromosome of *B. napus* would be 'safer' than on an A-chromosome (Metz et al. 1997; Lu et al. 2002). However, Tomiuk et al. (2000) states that the two genomes have close structural similarities which facilitate recombination between homologous A- and C-chromosomes in *B. napus* and in plants from backcrosses with *B. rapa*. *Brassica napus* specific DNA markers located on the C-chromosome were transferred to the BC₁ generation with *B. rapa* as the parent, indicating that integration of transgenes to the C-chromosome will not exclude transfer in interspecific cross, (Jørgensen et al. 1998).

521. This hypothesis is supported by the work of Stewart et al (2002). GM canola plants derived from twelve independent transformation events, presumably representing insertions in both the A and C genomes, were crossed with *B. rapa*. F1 hybrids backcrossed with *B. rapa* at similar rates.
522. In summary, *B. napus* and *B. rapa* occur in close proximity and there is flowering synchrony hybridisation and introgression will be possible. The rate of hybridisation and introgression will be influenced by the distribution, proximity and genetic compatibility of each species. Hybrids may have reduced fertility, seed set and fitness relative to their parents, however recent evidence suggests that hybrids may have increased female fitness and these factors will also be influenced by the frequency of parents and hybrids.

Brassica juncea

523. *Brassica juncea* has been reported as a weed in Queensland, New South Wales, Victoria, South Australia and Western Australia (Groves et al. 2000). However this species is only regarded as a minor problem in agricultural areas in New South Wales and Victoria where it has been grown commercially and does not occur as a weed of undisturbed natural habitats (P. Salisbury pers. comm. Salisbury 2002). *Brassica juncea* is grown on a small scale in Australia for the condiment and cold pressed oil markets, however, canola-quality *B. juncea* cultivars are likely to be commercially released in Australia in the next few years (Oram et al. 1999). *Brassica juncea* has a greater tolerance to heat and drought and is better suited to the drier areas of Australia than *B. napus*.
524. *Brassica juncea* shares a common set of chromosomes with canola and is self-compatible. In trials where *B. juncea* plants were planted in a canola field, 3 % of the *B. juncea* seeds were hybrids (Jørgensen et al. 1996). Bing et al. (1991) also reported 3 % hybridisation in the field when *B. napus* was the male parent. Crosses can occur in both directions, but hybrids with *B. napus* as the female were less successful (Jørgensen et al. 1998). Interspecific hybrids have reduced fertility (0-28 % pollen viability) and low seed set (Bing et al. 1991; Frello et al. 1995). *Brassica napus* specific DNA markers were transferred to the BC1 generation with *B. juncea* as the parent, indicating that backcrossing and subsequent introgression of *B. napus* genes could occur (Jørgensen et al. 1998).

Other Brassica species

525. Although *B. napus* and *B. oleracea* share a common set of chromosomes which makes hybridisation potentially possible, crosses have been difficult to generate even in laboratory conditions. (Eastham & Sweet 2002; Salisbury 2002). No hybrids have been reported in the field for *B. napus* and *B. oleracea* vegetables such as cauliflower, Brussel sprouts, broccoli, several kales, kohlrabi etc (Scheffler & Dale 1994). Unless used as a seed production crop, *B. oleracea* vegetables are generally harvested before flowering thereby limiting the potential for herbicide resistance genes to be acquired (Salisbury 2002). Furthermore, these plants are not recognised as weeds in agricultural environments in Australia.
526. *B. tournefortii* or *B. fruticulosa* are reported as problematic weeds in most States of Australia (Groves et al. 2002), however natural hybridisation between *B. napus* and either species has not been demonstrated and has only been achieved with hand crosses under glasshouse conditions (Scheffler & Dale 1994; Salisbury 2002).

2.2.3 Introgression of genes into other Brassicaceae species

527. Hybrids between canola and a number of Brassicaceae species have been reported following sophisticated hand pollination and embryo rescue techniques (Scheffler & Dale 1994; Salisbury & Wratten 1997; Rieger et al. 1999; OGTR 2002). However, this does not give an accurate indication of the potential for cross-pollination and introgression in the field.
528. Spontaneous cross pollination with related Brassicaceous species has been recorded, either in Australia or overseas, for three economically important weed species in Australia: *Raphanus raphanistrum*; *Hirschfeldia incana* and *Sinapis arvensis* (Salisbury 2002; Norris & Sweet 2003). The potential for transgene introgression in these species is discussed in detail below.

Raphanus raphanistrum

529. *Raphanus raphanistrum* (wild radish) occurs in Queensland, New South Wales, Victoria, Tasmania, South Australia and Western Australia (Groves et al. 2000). It is a major weed of cropping regions, particularly in southern Australia. Large numbers of *R. raphanistrum* can occur along roadsides and railway lines in and around canola growing areas in Australia (Agrisearch 2001; Dignam 2001). When surveyed by phone, weed personnel from National Parks in canola growing regions of Australia did not report *R. raphanistrum* as a weed unless prompted (Dignam 2001).
530. It should be noted that *R. raphanistrum* has a natural tolerance to glufosinate ammonium in the Australian environment (Kumaratilake et al. 2002) and therefore the transfer of the glufosinate ammonium tolerance gene would not alter the options for control of this weed.
531. Hybrids between canola and *R. raphanistrum* (wild radish) have been reported in the field both in Australia (Rieger et al. 2001; Rieger et al. 1999) and overseas (Darmency et al. 1995; Chevre et al. 1996; Chevre et al. 1997; Chevre et al. 1998; Darmency et al. 1998; Chevre et al. 1999; Chevre et al. 2000a; Chevre et al. 2000b; Warwick et al. 2003). *R. raphanistrum* is self-incompatible and therefore open to fertilisation from other pollen sources (Sampson 1967).
532. Natural interspecific crossing can occur in both directions between canola and *R. raphanistrum* but the rate of outcrossing varies with the direction of the cross. The frequency of hybrids is lower when canola is the pollen donor (Eber et al. 1994; Darmency et al. 1995; Chevre et al. 1996).

***B. napus* (male) x *R. raphanistrum* (female)**

533. When *R. raphanistrum* was grown in fields of canola in France, Chèvre et al. (1999; 2000a) reported estimated hybrid frequencies of 3×10^{-5} to 3×10^{-7} with canola as the pollen donor.
534. A study by Darmency et al (1998) identified 2 hybrids (from the same plant) from pollination of *R. raphanistrum* by chlorosulfuron-tolerant *B. napus* in field experiments from 1421 seeds screened in 1994, however no hybrids were detected in similar experiments in 1995 and 1996 (3804 seeds screened). These hybrids exhibited very low male fertility, with most flowers having aborted anthers and an average 0.5 pollen grains per flower (Benabdelmouna et al. 2003). Backcrossing the F1 hybrid by hand pollination with *R. raphanistrum* pollen revealed very low female fertility (0.18 seeds per 100 flowers) and the viability of resultant seeds was poor (Darmency et al. 1998; Benabdelmouna et al. 2003). The F1 hybrids had 28 chromosomes comprised of

addition of the haploid genomes of *R. raphanistrum* (Rr, n = 9) and *B. napus* (AC, n = 19) while the BC1 progeny had between 45 – 48 chromosomes, 9 contributed by *R. raphanistrum* with 36 – 39 from *B. napus*. Benabdelmouna et al. (2003) concluded that “the low seed set, absence of intergenomic recombination between the AC and Rr genomes, the apparent separate behaviour of the two sets of chromosomes, and the production of a complex karyotype could combine to result in a very low frequency of transgene introgression from *B. napus* to *R. raphanistrum*”.

535. Warwick et al (2003) also investigated the the incidence of hybrids of glyphosate tolerant *B. napus* (as pollen donor, male) and *R. raphanistrum* (as pollen recipient, female) in Canada, both in field plot experiments and in commercial canola fields. F1 hybrids were identified by glyphosate tolerance.
536. In two 10m x 10m experimental field plots *R. raphanistrum* at 1 plant/m² was co-cultivated with *B. napus* sown at commercial density as well as *R. raphanistrum* plants on the plot margin (0.5m from the plot and 1m apart). Only one hybrid was detected from 32,821 *R. raphanistrum* seeds screened in the field plot experiments, representing a hybridisation frequency of 3×10^{-5} (Warwick et al. 2003). The hybrid resembled *R. raphanistrum* and had a chromosome number of $2n = 37$ consistent with a genotype of RrRrAC resulting from the fusion of an unreduced gamete of *R. raphanistrum* (RrRr, $2n = 18$) with a reduced gamete of *B. napus* (AC, n = 19). The authors considered that “such a genotype was clearly unstable”. The hybrid was virtually male sterile with 0.12% pollen viability and did not set seed when self-pollinated (Warwick et al. 2003).
537. No hybrids were detected from 22,114 *R. raphanistrum* seeds collected in or near commercial glyphosate tolerant canola crops (Warwick et al. 2003).
538. Norris and Sweet (Norris & Sweet 2003) surveyed several sites in the UK over six years for hybrids of glufosinate ammonium tolerant GM canola and *R. raphanistrum* but found no evidence of hybridisation.
539. In an Australian study in which *R. raphanistrum* were planted into large plots of canola, no hybrids were detected amongst 25,000 seedlings grown from seed collected from the wild radish plants (Rieger et al. 2001). This represents a maximum rate of outcrossing of less than 4×10^{-5} with canola as the pollen donor.

***B. napus* (female) x *R. raphanistrum* (male)**

540. With male sterile canola as the pollen recipient, estimates of hybrid frequencies from 5×10^{-4} to 2×10^{-5} have been reported (Chevre et al. 1999; Chevre et al. 2000a). When male sterile canola is used as the pollen recipient, the frequency of interspecific hybrids increases (Eber et al. 1994; Darmency et al. 1995; Chevre et al. 1996). Darmency et al. (1995) reported that although hybrids grew as well as normal wild radish plants, they produced only 0.16 seeds per plant. This is compared to nearly 2200 seeds produced by a single wild radish plant. Therefore the relative fitness of hybrids compared to wild radish, in terms of viable seed produced was less than 0.01 %.
541. Further studies in France on *R. raphanistrum* (male) x *B. napus* (female) under field conditions have demonstrated that the hybrids showed significantly reduced fitness in comparison to either parent in two separate years (Gueritain et al. 2003a). These F1 hybrids showed lower and delayed seedling emergence and a lower survival than either parent. Most seedlings of the two parent species survived but around half of the hybrids died. Only 36% of the hybrids flowered compared to 81% for the parents and the time from emergence to flowering was significantly increased for the hybrid relative to either parent. Plant development in the hybrids was very reduced relative to

- both parents under conditions of competition. The authors concluded that the results imply that interspecific hybrids between *B. napus* and *R. raphanistrum* ate less likely than both parents to emerge and survive to reproduction under agronomic and natural conditions (Gueritaine et al. 2003a).
542. Several studies have demonstrated that there is significant variation between cultivars of canola and *R. raphanistrum* genotypes in terms of hybridisation (Baranger et al. 1995; Gueritaine & Darmency 2001; Gueritaine et al. 2003b). Gueritaine et al. (2001) reported polymorphism within a single population of *R. raphanistrum*. These genotypic variations affect prezygotic barriers to interspecific hybridisation such as the ability of *B. napus* to accept *R. raphanistrum* pollen and the rate of fertilization of ovules (Gueritaine & Darmency 2001; Gueritaine et al. 2003b).
543. In Australia, using non-GM herbicide tolerant canola, Rieger et al. (2001) found the frequency of hybridisation of *R. raphanistrum* into fertile canola to be 4×10^{-8} , detecting two hybrids from 52 million canola seedlings. The pollen viability of the hybrids (63 and 64 %) was comparable to *B. napus* and *R. raphanistrum* with an average of 58 and 71 %, respectively (Rieger et al. 2001). Both hybrids were capable of producing seed via selfing. This study investigated hybridisation using a mixture of 10 distinct *R. raphanistrum* populations.
544. A study in France by Pierre (2001) has suggested that honeybees (*Apis mellifera*) exhibit a significant preference for visiting canola flowers over *R. raphanistrum* flowers. The discrimination, although less, was also noted for the bumble bee species *Bombus terrestris* but *B. lapidarius* was more constant to *R. raphanistrum*. Small insects such as flies and solitary bees (not *Apis mellifera*) either showed a preference for *R. raphanistrum* or visited both species equally. Observations of pollen and nectar production indicated that *R. raphanistrum* was a less rewarding food source than canola. These observations may have relevance to the Australian situation where honeybees may be the main pollinators of canola.
545. Since hybridisation is more likely with *R. raphanistrum* pollinating *B. napus*, hybrid individuals are most likely to occur in crops, with the majority of seed removed at harvest (Rieger et al. 2001). However, seed generated from various crosses with male sterile canola lines and *R. raphanistrum* indicate a size dimorphism (Baranger et al. 1995a; Baranger et al. 1995b). Large seeds (diameter >1.6mm) belonged to *B. napus* (due to pollen contamination) and had a genomic constitution consistent with *B. napus* (AACC). Small seeds with a diameter =1.6mm gave rise to plants that were triploid hybrids (ACRr) with some amphidiploids (AACCRrRr), as well as normal diploids (AACC) and haploids (AC). If hybrids formed between fertile InVigor[®] canola and *R. raphanistrum* also have small seeds and these are not collected at harvest, glufosinate ammonium tolerant hybrid seeds could remain in the field. Any glufosinate ammonium tolerant hybrids remaining in the field following a InVigor[®] canola crop would be just as susceptible as InVigor[®] canola volunteers and would be readily controlled by a variety of herbicides and cultural control methods (see Appendix 4).
546. Hybrid seed can survive in the soil for at least 3 years (Chadoeuf et al. 1998). The viability of hybrid and *B. napus* seeds was determined in French fields that underwent deep ploughing and were then used as in a conventional farming system. Average germination of *B. napus* was 7 % after 1 year and 2 % at 3 years. Hybrid seeds declined in the same manner, but were around 1 % after the first year and less than 0.1 % after 3 years.

547. Overseas studies using glufosinate ammonium-tolerant GM canola have shown that fertility is low after backcrossing hybrids into *R. raphanistrum* (less than one backcross seed per plant, Darmency et al. 1995). Fertility was improved in subsequent backcross generations with *R. raphanistrum*, however the percentage of herbicide tolerant plants decreased (Chevre et al. 1997; Chevre et al. 1998). Chèvre et al. (1998) demonstrated that it is possible under field conditions to obtain glufosinate ammonium-tolerant plants close to *R. raphanistrum* in three generations. However, no stable canola introgression within the *R. raphanistrum* genome has been observed. After four generations of backcrossing to *R. raphanistrum*, and selecting herbicide tolerance in each generation, all herbicide tolerant plants contained one or more extra chromosomes, indicating that the herbicide tolerance gene from canola was not incorporated in the *R. raphanistrum* genome (Chevre et al. 1999).
548. Gueritain et al. (2002) recently examined the fitness of the backcross 6 (BC6) generation under field conditions. The BC5 generation was derived from an original F1 hybrid from a *R. raphanistrum* (pollen donor) x glufosinate-tolerant canola (female) cross backcrossed with *R. raphanistrum* as pollen donor, ie the BC5 hybrids have canola cytoplasm. BC6 plants with *R. raphanistrum* as pollen donor have canola cytoplasm, termed OBC (oilseed rape backcross), and those where the BC5 hybrid is the pollen donor to *R. raphanistrum* have *R. raphanistrum* cytoplasm, termed RBC (radish backcross). They found that the fitness value of the OBC plants was 100 times lower than for RBC plants based on plant growth, flowering and seed production. The RBC plants behaved similarly to *R. raphanistrum*. They also found that the bar gene was inherited at a lower rate than the 1:1 ratio predicted for a dominant Mendelian trait, however this phenomenon may be related to the particular chromosome on which the transgene is located (Gueritain et al. 2002).
549. Downey (1999a; 1999b) reported that French scientists have found significant barriers to the introgression of *B. napus* genes into the genome of *R. raphanistrum*. Although Chevre et al. (2000a) concluded that the transgene had not been introgressed through recombination into *R. raphanistrum*, Salisbury (2002a) reported that Chevre considered the stabilisation of hybrids with an intermediate number of chromosomes possible. Despite variations in observed rates, evidence from various research groups supports the conclusion that hybridisation between *B. napus* and *R. raphanistrum* occurs at very low rates, and that the resultant hybrids generally have significantly reduced reproductive fitness.

Hirschfeldia incana

550. *Hirschfeldia incana* (Buchan weed) occurs in Queensland, New South Wales, Victoria, Tasmania, South Australia and Western Australia and is characteristically a weed of disturbed soils in eastern Australia (Salisbury 2002). It is listed by Groves et al (2000) as a minor problem in agricultural areas of Queensland and New South Wales. *H. incana* is not permitted entry into Western Australia under the Permitted and Prohibited list of the Plant Diseases Act 1974 (Western Australia) and control is required in part of South Australia (The National Weeds Strategy 2003). *H. incana* is also capable of invading disturbed native vegetation. It can also occur in large numbers along railways and roadsides in canola growing regions in Australia (Dignam 2001).
551. Spontaneous hybridisation between canola and *H. incana* has been reported by a number of researchers. The rate of hybridization in the field is extremely variable but the mechanisms underlying this variation are still largely unknown. Some studies report low rates: 0.6 hybrids/plant (Darmency and Fleury 2000); while others report

much higher values especially when using male sterile *B. napus* (Lefol et al. 1991; Eber et al. 1994; Chevre et al. 1996; Lefol et al. 1996a). For example, between 1.5 – 26 hybrids per plant were recorded following an insect-proof caged experiment between *H. incana* and male sterile *B. napus*. The higher rates of hybridisation were found when female plants were at a lower density (1 plant per 12m²). However, hybrids were been shown to have reduced numbers of flowers, pods per flower, seeds per pod, and fewer seeds per plant than the *H. incana* parental type. In addition, as the density of *H. incana* increased, the fecundity of hybrids decreased (Lefol 1996). From a persistence and risk management perspective the rate of introgression into the recipient population is arguably of more consequence than the rate of gene transfer. From multi-generational studies, gene introgression did not occur even after 5 generations of backcrossing to *H. incana* (Darmency & Fleury 2000; Darmency 2001).

552. In summary, introgression of GM canola into *H. incana* is unlikely for two main reasons. Firstly, hybrids have low fertility and fitness relative to the parents, and secondly because of sexual incompatibility between canola and *H. incana* (Lefol et al. 1996b; Chevre et al. 1999). A gene in *H. incana* inhibits homeologous pairing (Lefol et al. 1996b), resulting in rapid expulsion of canola chromosomes in hybrids with *H. incana* (Salisbury 2002).

Sinapis arvensis

553. *Sinapis arvensis* (charlock) occurs in Queensland, New South Wales, Victoria, Tasmania, South Australia and Western Australia. For the most part, charlock is a problem in agricultural areas and is a particularly serious weed in cropping regions of New South Wales (Groves et al. 2000). It can also occur in disturbed sites along roadsides and railways in canola growing regions of Australia (Dignam 2001).
554. Hybridisation between *S. arvensis* and *B. napus* occurs at very low frequencies and the majority of studies have found embryo rescue or ovule culture to be the only methods of achieving hybridisation (Eastham & Sweet 2002). In a study with glufosinate ammonium-tolerant canola as the pollen donor, no hybrids were detected among 2.9 million seeds produced by *S. arvensis*, suggesting an outcrossing rate of less than 3×10^{-7} (Lefol et al. 1996a). Chèvre et al. (1996) failed to obtain any hybrids using *S. arvensis* as the female.
555. Using hand pollination, Moyes et al. (1999) did not detect any hybrids with *B. napus* as pollen donor from 6000 flowers pollinated. They concluded that their results, together with those of Lefol et al. (1996a), indicated that the *B. napus* (pollen donor) to *S. arvensis* cross was incompatible (1999). However further glasshouse studies by Moyes et al. (2002) with *S. arvensis* seed collected from 102 populations across the UK, obtained one hybrid with *B. napus* as the pollen donor after 1127 hand-pollinations of *S. arvensis* flowers resulting in a rate of 0.0015 % of the potential seed output indicating that a cross in this direction is possible. However they were unable to detect any gene transfer from *B. napus* to *S. arvensis* in field studies where single *S. arvensis* plants were transplanted into plots of canola of different varieties, no hybrids were detected from the 10,000 plants that were grown from the seed collected from *S. arvensis* (Moyes et al. 2002).
556. When male sterile glufosinate ammonium tolerant canola was used as the pollen recipient, hybridisation was only detected at extremely low frequency (ie. 6 hybrid seeds from 50,000 flowers, Lefol et al. 1996a). The pod produced from each flower usually contains 15 to 25 seeds (Buzza 1979). Hybrids formed using hand pollination

of *B. napus* flowers with *S. arvensis* pollen were formed at very low rates, from undetectable to 0.0049 % of the total seed potential (Moyes et al. 2002, Moyes et al. 1999). Under open pollination conditions, Chevre et al. (1996) obtained 0.18 seeds per 100 flowers with *S. arvensis* as the pollen donor. Under the same conditions, *S. arvensis* produced 850 seeds per 100 flowers and *B. napus* produced between 1238 and 2390 depending on the variety. Of the hybrids produced, 83 % were male sterile and pollen viability did not exceed 30 %.

557. Moyes et al. (1999) noted that for hybridisation of *B. napus* by *S. arvensis* in the commercial field situation, most *B. napus* seed would be harvested.
558. Studies in Canada not detect any *B. napus* x *S. arvensis* hybrids from 43,000 seedlings sampled from commercial glyphosate canola fields (Warwick et al. 2003). Similarly, herbicide challenge of 3,800 *S. arvensis* seeds sampled in the UK from 9 field trial locations of glufosinate ammonium and glyphosate tolerant GM canola did not detect any hybridisation (Norris & Sweet 2003).
559. Since the chance of an inserted gene being integrated into *S. arvensis* is extremely remote (Bing et al. 1991; Eber et al. 1994; Chevre et al. 1996; Lefol et al. 1996b; Moyes et al. 2002), no gene flow is likely to occur between canola and *S. arvensis* in the field (Downey 1999a; Downey 1999b).

Other weedy species in the Brassicaceae family

560. No natural hybrids between *B. napus* and other weedy species in the Brassicaceae family have been reported eg. *Brassica tournefortii*, *B. fruticulosa*; *B. oxyrrhina*, *Diplotaxis muralis*, *D. tenuifolia*, *Rapistrum rugosum* (Salisbury 1991). Even with the use of hand pollination and embryo rescue techniques, no hybrids have been obtained with weedy crucifer species in other tribes (eg. *Myagrum perfoliatum*, *Capsella bursa-pastoris*, *Sisymbrium* spp., *Cardaria draba* (Salisbury 1991; Salisbury 2002).

Section 2.3 Conclusions regarding gene transfer to other plants

Brassica napus vegetables and forage rape

561. The likelihood of gene transfer and introgression into *B. napus* vegetables (such as swedes, rutabaga and Siberian kale) or *B. napus* forage rape is very low. Gene transfer would require flowering synchrony and *B. napus* vegetables are generally harvested before flowering. *B. napus* vegetable seed production crops are isolated from other canola or *B. napus* vegetable crops to prevent outcrossing. Similarly forage rape crops flower rarely and are consumed prior to flowering or seed production.
562. Gene transfer from the glufosinate ammonium tolerant GM canola to *B. napus* vegetables or forage rape will not result in adverse impacts to human health and safety or the environment. The risk associated with gene transfer is therefore concluded to be negligible and no management conditions are required for this release.

Other Brassica species

563. In summary, *B. napus* and *B. rapa* occur in close proximity and there is flowering synchrony hybridisation and introgression will be possible. The rate of hybridisation and introgression will be influenced by the distribution, proximity and genetic compatibility of each species. Hybrids may have reduced fertility, seed set and fitness relative to their parents, however recent evidence suggests that hybrids may have

- increased female fitness and these factors will also be influenced by the frequency of parents and hybrids.
564. The likelihood of transfer of the introduced genes to the closely related *B. rapa* is high. The rate of hybridisation and introgression will be influenced by the distribution, proximity and genetic compatibility of each species. However, the frequency of outcrossing is expected to be even lower than for conventional (non-GM) canola because of the lower incidence of these species. Hybrids may have reduced fertility, seed set and fitness relative to their parents, however recent evidence suggests that hybrids may have increased female fitness and these factors will also be influenced by the frequency of parents and hybrids. Due to the greater incidence of *B. rapa* in Tasmania than on the mainland, gene transfer and introgression may be more likely to occur in Tasmania. However it should be noted that the main incidence of *B. rapa* is concentrated in particular geographic locations. If gene transfer did occur, glufosinate ammonium tolerant *B. rapa* could be managed using the same control measures as are currently used for control of Brassicaceous weeds, ie. herbicides and cultivation practices.
565. Transfer of the introduced genes from glufosinate ammonium tolerant GM canola to *B. rapa* will not result in adverse impacts to human health and safety or the environment. The risk associated with gene transfer is therefore concluded to be very low and no management conditions are required for this release.
566. The likelihood of transfer of the introduced genes to the closely related *B. juncea* is high. The rate of hybridisation and introgression will be influenced by the distribution, proximity and genetic compatibility of each species. However, the frequency of outcrossing is expected to be even lower than for conventional (non-GM) canola because of the lower incidence of these species and the reduced fitness of any hybrid progeny. If gene transfer did occur, glufosinate ammonium tolerant *B. juncea* could be managed using the same control measures as are currently used for control of Brassicaceous weeds, ie. herbicides and cultivation practices.
567. Transfer of the introduced genes from glufosinate ammonium tolerant GM canola to *B. juncea* will not result in adverse impacts to human health and safety or the environment. The risk associated with gene transfer is therefore concluded to be negligible and no management conditions are required for this release.
568. The likelihood of gene transfer and introgression from the GM canola into *Brassica oleracea* vegetables is negligible. *B. oleracea* is not considered a weed in Australia. Outcrossing from canola (conventional or GM) to *B. oleracea* is unlikely to occur as hybrids are not readily formed and commercial *B. oleracea* crops (eg. cabbage) are harvested prior to flowering. The risk associated with gene transfer from glufosinate ammonium tolerant GM canola to *B. oleracea* is concluded to be negligible and no management conditions are required for this release.

Brassicaceous weeds

569. The likelihood of gene transfer into weedy Brassicaceae species is extremely low because of the low frequency with which interspecific hybridisation occurs. Only three related species in Australia are considered as possible candidates for hybridisation and introgression – *R. raphanistrum*, *H. incana* and *S. arvensis* (Salisbury 2002), but for other brassicaceous species the possibility is considered negligible.

570. Inter-specific crossing between canola (either conventional or GM) and *R. raphanistrum* occurs at extremely low levels. The frequency of hybridisation is lower when canola is the pollen donor, hybrids are most likely to occur in canola crops with the majority of seed removed at harvest. Inter-specific hybrids of conventional canola with *R. raphanistrum* have low vigour and fertility. Even if outcrossing occurs, evidence suggests that there are significant barriers to introgression of genes from canola to *R. raphanistrum*.
571. *R. raphanistrum* has a natural tolerance to glufosinate ammonium in the Australian environment and therefore the transfer of the glufosinate ammonium-tolerance gene would not alter the options for control of this weed.
572. Inter-specific crossing between canola (conventional or GM) and *H. incana* is very unlikely to occur. Inter-specific hybrids of conventional canola with *H. incana* have low vigour and fertility. *H. incana* possesses genes that inhibit homeologous pairing of chromosomes resulting in the expulsion of *B. napus* chromosomes in inter-specific hybrids.
573. Inter-specific crossing between canola (conventional or GM) and *S. arvensis* is very unlikely to occur. Inter-specific hybrids of conventional canola with *S. arvensis* have low vigour and fertility.
574. If outcrossing and introgression of the introduced genes from the GM canola lines to *R. raphanistrum*, *H. incana* or *S. arvensis* did occur, the inter-specific hybrid plants would not have any survival advantage in the absence of glufosinate ammonium herbicide. Glufosinate ammonium is not widely used and is not registered for use in broad-acre agriculture. Glufosinate ammonium tolerant inter-specific hybrids can be effectively controlled with the herbicides and other non-chemical management techniques currently used.
575. The likelihood of transfer and introgression of genes from the glufosinate ammonium tolerant GM canola to *R. raphanistrum*, *H. incana* or *S. arvensis* is very low and would not result in adverse impacts to human health and safety or the environment. The risk associated with gene transfer is therefore concluded to be very low and no management conditions are required for this release.

2.3.2 Weediness risk

576. The introgression of glufosinate ammonium-tolerant genes from canola into other Brassica species or weedy relatives will not provide these plants with an ecological advantage.
577. The presence of a herbicide tolerance trait in plants does not confer a competitive advantage unless the specific herbicide is applied. Glufosinate ammonium tolerance in hybrid populations could only lead to an agronomic weed problem if an agricultural system relies substantially on glufosinate ammonium herbicide for weed control.
578. In the case of *B. rapa* and *B. juncea*, results indicate that hybrids will behave in a similar fashion to their parents in the absence of any herbicide selection. In the case of hybridisation with the related brassicaceous weedy species *R. raphanistrum*, *H. incana* and *S. arvensis*, the hybrid progeny of such crosses suffer significant reductions in reproductive fitness and competitive ability mitigating against any increased weediness as a result of gene transfer from the GM glufosinate ammonium tolerant canola.

579. In broad acre agriculture glufosinate ammonium has been registered as Liberty[®] for use only in InVigor canola crops and it would not be used for volunteer control. Therefore any hybrids with glufosinate ammonium tolerance can be managed using the same control measures as are currently used for control of Brassicaceous weeds, ie herbicides and cultivation practices.
580. Glufosinate ammonium-tolerant weeds would only have a selective advantage in situations where glufosinate ammonium is used for weed control. *R. raphanistrum* already has a natural tolerance for glufosinate ammonium. In Australia, the use of glufosinate ammonium is registered as Basta[®] for weed control is mainly limited to areas where grapevines, fruit trees and vegetables are grown. In the unlikely event that glufosinate ammonium tolerance is introgressed into weeds in these situations, there is a wide range of alternative herbicides and cultural practices available to control these plants.
581. Although glufosinate ammonium is registered under the tradename Finale[®] for weed control in areas associated with agricultural non-crop areas, commercial and industrial areas and rights-of-way, it is not widely used in these areas (Dignam 2001). Control of glufosinate ammonium-tolerant weeds in these areas would be readily achieved by the application of the herbicide usually used in their situation as well as on a wide range of other herbicides.
582. The risk of glufosinate ammonium-tolerant hybrid populations threatening undisturbed natural habitats is negligible since Brassicaceous weeds do not tend to invade and persist in natural undisturbed habitats in Australia (see Appendix 4). In addition, the introgression of glufosinate ammonium tolerance genes would not give these plants a selective advantage, as glufosinate ammonium is not registered for use in these locations.
583. Transfer of the barnase (male sterility), barstar (fertility restorer) or nptII (antibiotic resistance) genes to other plants would not confer a selective advantage. Although some of the regulatory sequences are derived from a plant pathogen (*A. tumefaciens*, cauliflower mosaic virus), they only represent a very small proportion of the pathogen genome and are not in itself pathogenic or infectious.

2.3.3 Conclusions

584. The conclusions with respect to the specific transferred gene sequences are as follows:
- Herbicide tolerance gene: The frequency of outcrossing to other *Brassica* species and weedy *Brassicaceae* species is low to extremely low. There would be no adverse consequences even if outcrossing occurs, since these plants will only have a selective advantage in the presence of glufosinate ammonium. Glufosinate ammonium is not registered for use in broad acre cropping and is not used widely in uncropped disturbed habitats.
 - Male sterility gene: In the unlikely event that this gene was to be transferred, unless pollinated, these plants cannot reproduce and persist in the environment. In addition, the proportion of male sterile plants would decrease in subsequent generations in the absence of selective pressure.
 - Fertility restorer gene: In the unlikely event his gene was transferred, it would have no impact on a plant's phenotype apart from restoring male fertility to a portion of the progeny of plants with the male sterile gene.

- Antibiotic resistance gene: This gene would not confer a selective advantage to other plants.
- Promoter and other regulatory sequences: The probability of a hazard arising due to outcrossing of these sequences to other plants is remote, given the low likelihood of gene transfer occurring. Plants are already exposed in nature to the bacteria from which these sequences are derived. Although some of the regulatory sequences are derived from plant pathogens (*A. tumefaciens*, Cauliflower Mosaic Virus), they only represent a very small proportion of the pathogen genome and are not, in themselves, infectious or pathogenic.

SECTION 3 TRANSFER OF INTRODUCED GENES TO OTHER ORGANISMS (MICROORGANISMS & ANIMALS)

Section 3.1 Nature of the gene transfer hazard

3.1.1 Mechanisms of horizontal gene transfer

585. Transfer of the introduced genes to other organisms (microorganisms and animals) could only happen as a result of horizontal gene transfer (non-sexual, non-parental-to-offspring gene transfer). There are no known mechanisms whereby horizontal gene transfer could occur between plants and mammalian cells, therefore primary consideration will be given to the possibility of transfer from GM plants to microorganisms. In bacteria, three mechanisms of horizontal gene transfer (HGT) have been described: transduction, conjugation, and transformation.
586. Transduction is a bacterium-virus interaction that can mediate gene transfer between bacteria in the environment (e.g. on plant leaf surfaces, in soil or water). Viruses that function in more than one species are known, but viruses that function in both plants and bacteria, and thereby facilitate HGT from plants to bacteria have not been identified (Nielsen et al. 1998).
587. Conjugation is a mechanism of cell-to-cell interaction that can mediate gene transfer between bacteria in the environment (e.g. in soil, on plant surfaces, in water etc). Conjugation is known to occur frequently between compatible bacteria with the transferable genes usually residing on plasmids. Transfer of chromosomal genes is much less frequent, except for some high frequency recombination strains. Conjugative gene transfer has been regarded as the most frequently occurring mechanism of HGT between bacteria (Sprague, Jr. 1991; Amabile-Cuevas & Chicurel 1993; Dreiseikelmann 1994; Souza & Eguiarte 1997). However, mechanisms that support conjugative gene transfer from higher plants to bacteria are not known (e.g. transposons that function in both plants and prokaryotes) (Nielsen et al. 1998).
588. Gene transfer by transformation is a process that allows bacteria, which are able to express a regulated physiological state of competence, to take up and integrate free DNA from their surroundings. This has been shown to occur in environments such as in soil, on plants, and in water. Most studies describing natural transformation have been conducted in vitro (Streips 1991; Lorenz & Wackernagel 1994) but often are of little relevance to most natural terrestrial environments. Natural transformation is regarded as the most likely mechanism whereby genes may move horizontally from GM plants to other organisms.

3.1.2 Potential hazards of transfer of the genes from the GM canola

589. All of the genes present in the GM canola lines are derived from commonly occurring bacteria. As detailed in Appendices 2 and 3, the proteins produced by the introduced genes are not considered toxic or allergenic.

Herbicide tolerance gene (*pat/bar* genes)

590. The herbicide-tolerance genes *pat* and *bar* were originally isolated from the common soil bacteria *Streptomyces viridichromogenes* and *S. hygroscopicus*, respectively, which are not considered pathogenic to plants, humans or other animals (Organisation for Economic Co-operation and Development (OECD) 1999). These genes are already present naturally in the environment. Transfer of these genes would not present a hazard to human health or the environment.

Male sterility gene (*barnase*) and the fertility restorer gene (*barstar*)

591. These genes are derived from *Bacillus amyloliquefaciens* and the *barnase* gene encodes an RNase and the *barstar* gene encodes its inhibitor (see Appendix 1 for details). RNases are ubiquitous in nature and serve many biological functions. *B. amyloliquefaciens* is a commonly occurring soil bacterium and is frequently used as a source for industrial enzymes such as alpha amylase (ANZFA 2001).

Kanamycin resistance gene (*nptII*)

592. The *nptII* gene was originally isolated from transposon Tn5 of *Escherichia coli*, a commensal bacterium of the human gut. The *nptII* gene and gene product are naturally present in bacteria in the environment and resistance to the antibiotics it inactivates is widespread. In addition, the antibiotics kanamycin and neomycin are not critical for the treatment of human disease. The consequences of transfer of the *nptII* gene from the GM canola lines would be negligible given that the *nptII* gene occurs naturally on transmissible genetic elements (transposons and plasmids) that are readily transferable between bacterial species (US FDA 1998; Flavell et al. 1992; Langridge 1997; Pittard 1997). Transfer of resistance is far more likely to occur from natural sources than from gene transfer from GM canola. Furthermore, the GM canola lines which Bayer proposes to commercialise, RF3 and MS8, do not contain the *nptII* gene.

Promoters and other regulatory sequences

593. If gene transfer occurred, there could be unintended or unexpected effects if the introduced regulatory sequences alter the expression of endogenous genes. If such perturbation of normal gene expression occurred, the impact would depend on the resultant phenotype.

594. The regulatory sequences present in the GM canola lines (see Appendix 1 for details) are derived from other plants (*Arabidopsis* and tobacco), *Agrobacterium tumefaciens* and cauliflower mosaic virus (CaMV). All of these sequences and the organisms they were derived from are frequently encountered in the environment. While some of these sequences are derived from plant pathogens (*Agrobacterium tumefaciens*, CaMV) the regulatory sequences only represent a very small proportion of the pathogen genome and are not, in themselves, infectious or pathogenic.

595. While Ho et al. (2000) have postulated that there are risks posed through recombination of the CaMV 35S promoter with the genomes of other viruses infecting the plants to

create new viruses, or of integration of the CaMV35S promoter into other species causing mutations, cancer or reactivation of dormant viruses, these claims have been challenged in the scientific literature (Hull et al. 2000; Morel & Tepfer 2000; eg Hodgson 2000b; Hodgson 2000c; Tepfer 2002). It should be noted that CaMV is already ubiquitous in the environment (Hodgson 2000a).

596. The GM canola line Topas 19/2 also contains a cos site of bacteriophage lambda, a COLE1 origin of replication from *E. coli*, and a supF suppressor tRNA gene from *E. coli* (data supplied by Bayer, European Scientific Committee on Plants 1998). These sequences are common and will only function in prokaryotes and there is no additional risk of transfer of these sequences than is already present due to the presence of these bacteria in the environment.

Section 3.2 Likelihood of the gene transfer hazard occurring

597. Gene transfer can occur between sexually incompatible organisms. Most gene transfers have been identified through phylogenetic analysis (Ochman et al. 2000; Smith & Oehme 1992; Worobey & Holmes 1999). In general, gene transfers are detected over an evolutionary time scale of millions of years (Lawrence & Ochman 1998; Doolittle 1999). Evidence from gene sequences indicate that, on a human time scale, transfer of genes between plants and other organisms such as animals, bacteria, fungi or viruses is exceedingly rare. Most gene transfers have been from virus to virus (Lai 1992), or between bacteria (Ochman et al. 2000). Less frequently, viruses have transferred genes to their hosts.
598. Theoretically, horizontal gene transfer from GM canola to other organisms, including humans and microorganisms is possible, but it is extremely unlikely.
599. This is because HGT does not happen frequently, as inferred from phylogenetic analyses, and because there are a number of barriers to horizontal gene transfer including temporal and spatial, biochemical, physiological, transfer, establishment, expression and evolutionary barriers (Nielsen 1998).
600. The transfer of plant genes to bacteria and viruses has been observed in laboratory and glasshouse experiments. However, in all cases this was achieved only under controlled conditions in the presence of related gene sequences (homologous recombination), and using highly sensitive or powerful selection methods to detect rare gene transfer events (see Section 3.2.3 for details).
601. The likelihood of hazard arising from gene transfer between plants and other organisms depends on the successful outcome of a series of individual events, including:
- survival of the genetic material in the soil or gut; and
 - opportunity for an organism or virus to encounter plant DNA or RNA and to take up that genetic material; and
 - evasion of efficient cellular defence mechanisms for degrading foreign nucleic acids; and
 - incorporation of the genetic material into the genome of the recipient organism or virus, at a site and in a configuration that allows the gene to be functional; and
 - persistence of the new gene in a stable configuration that allows the newly modified organism or virus to survive and reproduce; and

- significance of the transferred genetic material such that its presence and/or expression in the recipient organism will result in a hazard, ie adverse impacts on human health and safety, or the environment.

602. The likelihood of each of these events occurring is extremely low, and the combined probability of forming an unbroken chain of events resulting in a hazard is negligible.

3.2.1 Likelihood of gene transfer from GM plants to humans

603. The most obvious route of entry of foreign DNA into mammals is through food, as it passes through the gastrointestinal tract. The epithelial lining of the gastrointestinal tract has been considered akin to a monolayer culture of mammalian cells exposed to foreign DNA. Microorganisms colonise the whole length of the gastrointestinal tract, aiding the digestive process.

604. Canola oil is the only fraction of GM canola plants to be eaten as food by humans. Canola oil undergoes extensive processing and the oil does not contain any protein or nucleic acid. DNA was not detected in oil in any of GM canola lines (ANZFA 2001).

605. Since humans will not be exposed to DNA from the GM canola lines via the digestive system, the possibility of gene transfer to human cells or microorganisms in the human gut was not be considered further.

3.2.2 Likelihood of gene transfer from GM plants to animals

606. It is possible that GM canola plants may be consumed as forage or feed by farm animals. These animals and their associated microflora will be exposed to the transgenes of InVigor[®] GM canola and horizontal gene transfer is therefore possible, although unlikely.

607. Many bacteria, including representatives of the oral and gut microflora, are known to be naturally transformable. The possibility of transformation occurring in gut bacteria has received little attention, largely because free DNA has been considered unlikely to survive the action of high levels of pancreas-derived DNAase in the small intestine and other areas of the gut.

608. The possibility of DNA transfer in the gut has been investigated by feeding mice purified bacteriophage M13 DNA (Schubbert et al. 1997). Bacteriophage DNA was detected in the faeces and the livers of mice as well as in newborn mice (Schubbert et al. 1997). Only 1-2% of orally ingested bacteriophage DNA survived passage through the gastrointestinal tract of mice. However the relevance of this work to gene transfer from transgenic plants was questioned by Beever and Kemp (2000) who concluded that the bacteriophage DNA-containing cells in various organs were macrophages involved in scavenging and removing foreign DNA.

609. Alexander et al. (2002) recently investigated the digestive fate of DNA from GM glyphosate tolerant (Roundup Ready[®]) canola. They used PCR to detect the presence of two genes in various canola feed fractions following in vitro incubated in bovine ruminal fluid. The genes analysed were the CP4-EPSPS gene (which confers tolerance to the herbicide glyphosate) introduced by genetic modification and an endogenous nuclear-encoded rbcS gene (encoding the small subunit of the photosynthetic enzyme Rubisco).

610. Whole seed, cracked seed, canola meal or a 'diet' ration containing 6.5% canola meal were incubated in batch cultures of ruminal fluid. Processing of canola seed was found

to reduce the amount of DNA present, with the amount and integrity of DNA being significantly reduced in meal. There were no significant differences in the detection of the introduced or endogenous gene. Both genes could be detected in the cultures of whole and cracked seed for up to 48 hours, but only up to eight hours for whole meal and four hours for the fractional diet. Neither gene could be detected in the aqueous phase of the ruminal culture, but was detected in the plant debris. The authors concluded that the plant DNA was rapidly degraded by rumen fluid, and that the persistence of DNA was inversely related to plant cell digestion (Alexander et al. 2002). These results support the conclusion that the rapid degradation of DNA following release from plant cells during ruminant digestion represents a considerable barrier to transfer of plant DNA, GM and non-GM, to rumen bacteria or to ruminant animals.

611. Einspanier et al. (2001) investigated the fate of DNA from GM insect-resistant (Bt) maize fed to cattle and chickens by following the presence of the introduced cryIA(b) gene (which confers resistance to insects) and an endogenous chloroplast marker sequence using PCR. The chloroplast marker sequence resides on the chloroplast chromosome not in the nucleus and so is present in multiple copies in the GM maize relative to the cryIA(b) gene.
612. For cattle fed GM maize silage, both the cryIA(b) gene and the chloroplast marker were detected in chyme (duodenal juice). The chloroplast marker was detected in lymphocytes and faint signals were occasionally detected in milk, but it was not detected in faeces, whole blood, muscle, liver or spleen. The cryIA(b) gene was not detected in any of these samples (Einspanier et al. 2001).
613. In chickens fed a diet containing GM maize, the chloroplast marker was detected in muscle, liver, spleen and kidney, but not in faeces or eggs. In contrast, the cryIA(b) gene was not detected in any tissue sample or eggs (Einspanier et al. 2001).
614. A review of the safety issues associated with the DNA in animal feed derived from GM crops (Beever & Kemp 2000) indicated exposure to introduced DNA from GM crop material is negligible compared with normal exposure to non-transgenic DNA. They considered the impact of GM maize fed to dairy cows either as forage maize silage or maize grain. They calculated that, if the GM material comprises of 40% of the ration, in a 600 kg cow, transgene DNA consumption would amount to 2.6 µg/day. This compares to with a total diet DNA intake of 608 mg/day, equating to a ratio of GM DNA to normal plant DNA of 1:234,000 or 0.00042% of total dietary DNA.
615. Any uptake of plant DNA or RNA is likely to occur in non-reproductive (somatic) cells such as the lining of the gut. Even if gene transfer actually occurred, the gene would only be transferred to an individual cell, the introduced gene would not be transmitted in the germline to the progeny.
616. There is no evidence that the genes present in GM canola lines could be transferred from GM canola plants to animals, nor is there any evidence that the transfer of DNA from plants to animals has occurred during evolutionary history, despite the fact that animals eat large quantities of plant DNA.

3.2.3 Likelihood of gene transfer from GM canola to microorganisms

617. Transfer of the introduced genes from the GM canola lines to microorganisms is extremely unlikely.

Transfer to bacteria

618. Horizontal gene transfer from plants to bacteria has not been demonstrated under natural conditions (Syvanen 1999) and deliberate attempts to induce such transfers have so far failed (eg Schlüter et al. 1995; Coghlan 2000). Transfer of plant DNA to bacteria has been demonstrated only under highly artificial laboratory conditions, between homologous sequences and under conditions of selective pressure (Mercer et al. 1999; Gebhard & Smalla 1998; De Vries & Wackernagel 1998; De Vries et al. 2001) and even then only, at a very low frequency.
619. Uptake of DNA fragments extracted from transgenic plants by bacteria has been demonstrated in vitro and in artificial soil microcosms, based on restoration of a partially deleted bacterial kanamycin resistance gene (*nptII*) after recombination with transgenic plant-inserted homologues (Gebhard & Smalla 1999; Nielsen et al. 2000; De Vries & Wackernagel 1998). Without the artificially introduced homology in the recipient strain, no uptake of DNA could be detected in either *Acinobacter* sp. (Nielsen et al. 2000; De Vries et al. 2001) or *Pseudomonas stutzeri* (De Vries et al. 2001). Transformation of *Acinobacter* sp. with transgenic sugar beet DNA could not be detected in non-sterile soil microcosms (Nielsen et al. 2000). The relevance of such studies done under optimised in vitro conditions to natural systems such as soil is questionable.
620. The stability of released DNA in the terrestrial environment is essential for transformation to occur successfully. Several studies have demonstrated the persistence of plant DNA in the soil (Gebhard & Smalla 1999; Smalla et al. 1993). Long term persistence in soil of DNA from transgenic plants has been shown under field conditions for up to 2 years, and also for up to six months in soil microcosms where purified transgenic plant DNA was introduced (Gebhard & Smalla 1999). However no transgenic DNA could be detected in bacterial isolates from these soils (Gebhard & Smalla 1999).
621. Competence in bacteria is not usually constitutively expressed and bacterial cells that are transformable need to enter a physiologically regulated state of competence for the uptake of exogenous DNA (Lorenz & Wackernagel 1994). Non-competent *Acinetobacter* sp. in sterile soil microcosms could be induced to integrate a bacterial marker gene from transgenic sugar beet DNA by the addition of nutrients (Nielsen et al. 1997).
622. Studies have identified that plant DNA survives for some time in the animal digestive tract (Duggan et al. 2000; Einspanier et al. 2001; Aumaitre et al. 2002; Alexander et al. 2002; Duggan et al. 2003) and transfer to microbes in the animal or human gut may be a theoretical possibility. However there is no evidence of transfer of DNA from plants to bacteria in the digestive tract of humans or animals, including birds (Chambers et al. 2002).
623. Integration of genes into the genome of recipient bacteria is known to be dependent on sequence homology between the captured DNA and that of the recipient bacteria. It seems that heterology between these sequences is the main barrier to the stable introduction of diverged DNA in bacteria (Baron et al. 1968; Rayssiguier et al. 1989; Matic et al. 1995; Vulic et al. 1997). There is a decreasing exponential relationship between recombination frequencies in enterobacteria and increasing sequence divergence of the introduced DNA (Vulic et al. 1997). Although there is a higher probability of recombination when the sequences become more similar, the risks of

adverse effects resulting from such recombination is reduced because the likelihood of novel and hazardous recombinants being generated is less.

624. Even if transfer and establishment barriers were overcome, there are also barriers to expression of the exogenous genes. Gene promoters have to be compatible with expression in prokaryotes. Even if all of these steps were to occur, probably the single most important factor in determining whether the exogenous DNA would be integrated into bacteria is the strength of selection pressure. Prokaryotes have efficient genomes and generally do not contain extraneous sequences. If the genes are not useful to the organism then there will be no selective advantage in either integrating the genes or maintaining them in the genome.
625. All of the novel genes introduced into the GM canola lines are under the control of eukaryotic regulatory sequences (see Appendix 1 for details), therefore even if any of these genes were transferred to bacteria it is highly unlikely that they would be expressed.

Transfer to fungi

626. Fungi are known to be transformable and horizontal gene transfer from plants to plant-associated fungi has been claimed. Uptake of DNA from the host plant by *Plasmodiophora brassicae* (Bryngelsson et al. 1988; Buhariwalla & Mithen 1995) and uptake of the hygromycin gene from a GM plant by *Aspergillus niger* (Hoffman et al. 1994) have been reported. However, stable integration and inheritance of the plant DNA in the genome of these fungi has not been substantiated by experimental evidence (Nielsen 1998).

Transfer to plant viruses

627. There is a theoretical possibility of recombination between sequences that have been introduced into the genome of genetically modified canola and the genome of viruses that might infect the canola plants (Hodgson 2000a; Ho et al. 2000; Hodgson 2000c). Recombination between viral sequences and plant transgenes has only been observed at very low levels, and only between homologous sequences under conditions of selective pressure, eg regeneration of infectious virus by complementation of a defective virus by viral sequences introduced into a GM plant genome (Greene & Allison 1994; Teycheney & Tepfer 1999).

Section 3.3 Conclusions regarding gene transfer to other organisms

628. The likelihood of gene transfer from the GM canola plants to animals (including humans) or microorganisms is considered negligible because:
- Limited probability of occurrence. The likelihood of interaction, uptake and integration of intact plant DNA by other organisms occurring is negligible, especially if it involves unrelated sequences (non-homologous recombination);
 - Limited probability of persistence. The likelihood that any novel organism that does arise from gene transfer will survive, reproduce and have a selective advantage (competitiveness or fitness) is extremely low;
 - Natural events of horizontal gene flow from plants to distantly related organisms are extremely rare; and
 - Demonstration of horizontal gene transfer has generally been achieved only under highly controlled experimental conditions and with related gene sequences

(homologous recombination) using high selective pressure and sensitive detection systems to identify very rare events.

629. All of the introduced genes are derived from common bacteria and any organism that acquires the novel genes is unlikely to pose any additional risks to human health and safety, or the environment, compared to the GM canola lines.
630. The GM canola lines which Bayer propose to commercialise in Australia do not contain the *nptII* gene or sequence from cauliflower mosaic virus, therefore even the theoretical hazards postulated for the transfer of these sequences will not apply.

APPENDIX 6 HERBICIDE RESISTANCE

631. Under section 51 of the Act, the Regulator is required to consider risks to human health and safety and the environment in preparing the risk assessment and the risk management plan. In this part of the document, risks posed by the proposed dealing to the environment are considered in relation to the potential for the development of herbicide resistance among weeds.

SECTION 1 HERBICIDE RESISTANCE HAZARD

632. There is some potential for development of herbicide-resistant weeds if the InVigor[®] crop-Liberty[®] herbicide combination is used inappropriately.
633. The use of Liberty[®] herbicide (a formulation of glufosinate ammonium) on InVigor[®] canola crops in Australia is registered by the APVMA. The APVMA has responsibility for setting registration conditions for the use of glufosinate on canola crops, including implementation of herbicide resistance management programs.
634. InVigor[®] hybrid canola will be supplied through accredited resellers from 2004. Growers will be required to sign a grower agreement and will be trained to follow the InVigor[®] Canola Crop Management Plan (CMP). The CMP aims to ensure awareness of the industry protocols for coexistence of GM and other canola and knowledge of the regulatory conditions placed on the seed and herbicide. The APVMA is satisfied that implementation of the CMP will provide effective management of the development of herbicide resistance.

SECTION 2 CONCLUSIONS REGARDING HERBICIDE RESISTANCE

635. There is potential for development of herbicide-resistant weeds if the InVigor[®] crop-Liberty[®] herbicide combination is used inappropriately. This risk is managed by the APVMA, under conditions of registration for the use of agricultural chemicals in Australia. Therefore no specific conditions are imposed in the licence in relation to management of herbicide resistance, however the requirement to comply with conditions imposed by the APVMA is noted.

APPENDIX 7 INDUSTRY GUIDANCE MATERIAL

636. Considerable media and written communication has focussed on the possible impact of commercial release of GM canola on non-GM crops and markets eg. the status of Australian grain exports. It is important to note that evaluation of trade, marketing and cost/benefit issues have been intentionally excluded from the Gene Technology Act 2000 assessment process. Such issues were excluded because during consultations in the development of the legislation, governments formed the view that economic considerations should never be allowed to override the assessment of public health, safety and/or environmental risks. Therefore, this risk assessment and risk management plan cannot draw any conclusions about the possible costs or benefits of GM canola to farmers or the agricultural industry.
637. However, these issues are being actively considered by the Commonwealth, State and Territory Governments (both individually and through forums such as the Primary Industries Ministerial Council and its Plant Industries Committee) and by industry through the Gene Technology Grains Committee (GTGC). The GTGC Canola Industry Stewardship Protocols for Coexistence of Production Systems and Supply Chains and the applicant's InVigor[®] Crop Management Plan were both considered during the evaluation process to identify any additional possible risks posed by commercial release. However, the Regulator concluded that mixing and dissemination of GM canola in the supply chain would not pose any additional risks to human health and safety or the environment to the dealings proposed in the application, which do not anticipate any containment measures, such as buffer zones (i.e. the risk assessment process considered the risks that might occur in the absence of supply chain management controls). The key elements of associated documents are outlined below.

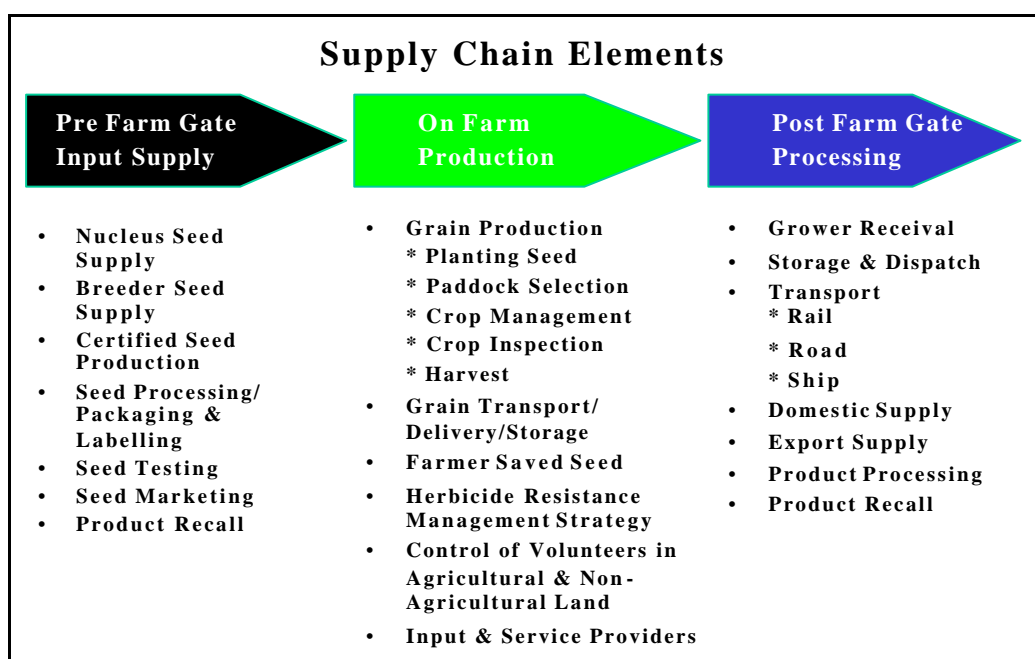
SECTION 1 PLANT INDUSTRIES COMMITTEE

638. The Primary Industries Ministerial Council (representing Commonwealth, State and Territory Governments) agreed, with the exception of Tasmania, that "management of GM risks to agricultural production by industry self-regulation supplemented by government monitoring". However, to assist the process, the Ministerial Council's Plant Industries Committee (PIC) prepared a document entitled Guidelines for Industry Stewardship Programs and Crop Management Plans for the Management of Genetically Modified Crops in Australian Farming Systems as a proposed set of indicative principles and circulated the document for public consultation in late 2002. This document identified a number of components which might be included in any industry stewardship program for on farm management of GM crops. The components identified by the PIC included:
- on-farm Crop Management Plan (CMP) which forms the foundation of the stewardship program;
 - communication and education;
 - compliance, auditing and enforcement;
 - reporting and assessment of agricultural and environmental impacts; and
 - contingency plans.
639. In addition the document suggested information that might be contained in crop management plans such as:
- on-farm record-keeping and documentation;

- crop hygiene including volunteer control, separation distances and seed quality; and
- herbicide tolerant crops including herbicide resistance management and out-crossing to related species.

SECTION 2 GENE TECHNOLOGY GRAINS COMMITTEE

640. The Gene Technology Grains Committee (GTGC) comprises representation from across the grains industry including producers, research institutions, technology providers, bulk handlers, food processors, the organics industry, farmer’s associations and observers from the State and Commonwealth Governments. The GTGC has produced a draft document entitled Canola Industry Stewardship Protocols for Coexistence of Production Systems and Supply Chains.
641. The Protocols are consistent with PIC’s indicative on-farm principles for industry (Gene Technology Grains Committee 2002) and describe various mechanisms with which all participants in the production and processing supply chain for canola can achieve ‘coexistence’ between GM and non-GM canola. The various components of the supply chain are detailed in the following diagram from the draft protocols document (p.4)



Gene Technology Grains Committee diagrammatic presentation of canola supply chain elements

642. The GTGC protocols provide advice and guidance to promote responsible crop hygiene and market access practice throughout the supply chain including: seed production and marketing; crop management plans; and receival, storage, handling and dispatch. These protocols have been issued for public comment. Copies may be obtained from AVCARE via their website: <http://www.avcare.org.au>

SECTION 3 BAYER'S STEWARDSHIP STRATEGY

643. In accordance with both the PIC and GTGC guidelines, Bayer has developed a stewardship strategy for InVigor[®] canola, underpinned by a crop management plan. The stated aims of the management recommendations in the InVigor[®] Crop Management Plan are to “ensure sustainability and efficacy in use; and enable growers to manage InVigor[®] hybrid canola within a system that allows the coexistence of alternative canola production systems”.
644. Under their stewardship strategy, Bayer proposes to educate all growers and agronomists/resellers about the standards for managing the technology. During the first two years of production, only those growers that have undergone training and have passed an accreditation test will be allowed access to InVigor[®] hybrid canola. Bayer proposes to review this requirement after two years, however it is anticipated that growers may nominate an accredited agronomist rather than completing the training and obtaining accreditation themselves.
645. To ensure compliance with the guidelines, Bayer proposes to audit growers, seed companies and seed distributors. Bayer's target number of audits for the first 4 years following approval are provided in Table 2.

Table 1: The minimum number of audits proposed by applicant during first 4 seasons following approval (assuming approval is granted in 2003)

Audit	Minimum % of growers audited			
	2003	2004	2005*	2006*+
<u>Grower</u>				
Grain delivery	0	5	5	5
Seed storage	0	5	5	5
Paddock following InVigor [®]	0	100	10	5
<u>Seed company</u>				
Seed production	100	100	100	100
Seed lot distribution	100	50	50	50
<u>Seed distributor</u>				
Seed distribution to Growers	50	25	10	10

*This procedure is valid until December 2004, with criteria for 2005 & later years to be reviewed at that time.

646. InVigor[®] hybrid canola will be supplied through accredited resellers from 2004. Growers will be required to sign a grower agreement and will be trained to follow the Crop Management Plan (CMP). The CMP aims to ensure awareness of the industry protocols for coexistence of GM and other canola and knowledge of the regulatory conditions placed on the seed and herbicide.

APPENDIX 8 LICENCE CONDITIONS AND REASONS FOR THE CONDITIONS

General Note

Note: Although there are no health, safety or environmental concerns that require continued monitoring by the OGTR, Bayer CropScience is obliged to comply with all relevant government requirements.

Note in relation to Herbicide Resistance Management

Note: The GMOs referred to in this licence are modified to confer tolerance to the herbicide glufosinate ammonium. The AVPMA has responsibility for setting registration conditions for the use of herbicides in Australia which may include the implementation of herbicide resistance management programs. Accordingly, it is not necessary for conditions in this licence to manage risks associated with the use of herbicides generally in connection with the GMOs.

PART 1 INTERPRETATION AND DEFINITIONS

Words and phrases used in this licence have the same meanings as they do in the *Gene Technology Act 2000* and the *Gene Technology Regulations 2001*.

Words importing a gender include any other gender.

Words in the singular include the plural and words in the plural include the singular.

Words importing persons include a partnership and a body whether corporate or otherwise.

References to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time unless the contrary intention appears.

Where any word or phrase is given a defined meaning, any other part of speech or other grammatical form in respect of that word or phrase has a corresponding meaning.

In this licence:

'Act' means the *Gene Technology Act 2000* (Cth).

'GM' means *genetically modified*.

'GMOs' means the genetically modified organisms covered by this licence, described at Attachment A.

'Material from the GMOs' means genetically modified material (including parts of GMOs) that are derived from or produced by the GMOs.

‘OGTR’ means the Office of the Gene Technology Regulator.

‘Regulator’ means the Gene Technology Regulator.

PART 2 GENERAL CONDITIONS

Duration of Licence

1. This licence remains in force until it is suspended, cancelled or surrendered. No dealings with GMOs are authorised during any period of suspension.

Holder of licence

2. The holder of this licence (‘the licence holder’) is Bayer CropScience Pty Ltd.

No dealings with GMO except as authorised by this licence

3. Persons covered by this licence must not deal with the GMOs except as expressly permitted by this licence.

Permitted dealings

4. The permitted dealings with the GMOs are all dealings with the GMOs.

Persons covered by this GMO licence

5. The persons covered by this licence are all persons in Australia.

Informing people of their obligations

6. The licence holder must inform any person covered by this licence, to whom a particular condition of this licence applies, of the following: the particular condition (including any variations of it); the cancellation or suspension of the licence; the surrender of the licence.
7. The licence holder must provide the Regulator, on the Regulator’s written request, signed statements from persons covered by this licence that the licence holder has informed those people of the conditions of this licence that apply to them.

<p><i>Note:</i> Condition 6 mirrors the statutory condition in section 63 of the Act. Under the Act, the Regulator may specify the manner in which information is to be provided to persons covered by the licence.</p>

Requirement to notify of circumstances that might affect suitability

8. The licence holder must immediately, by notice in writing, inform the Regulator of:
 - (a) any relevant conviction of the licence holder occurring after the commencement of this licence;

- (b) any revocation or suspension of a licence or permit held by the licence holder under a law of the Commonwealth, a State or a foreign country, being a law relating to the health and safety of people or the environment; or
- (c) any event or circumstances occurring after the commencement of this licence that would affect the capacity of the licence holder to meet the conditions in it.

Note: Section 57 of the Act prohibits the Regulator from issuing a GMO licence unless satisfied that the applicant is a suitable person to hold the licence. This condition ensures that the Regulator is notified if there are changes to relevant factors affecting a licence holder's suitability to hold a licence.

Additional information to be given to the Regulator

9. The licence holder must immediately notify the Regulator in writing if he or she:
- (a) becomes aware of additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or
 - (b) becomes aware of any contraventions of the licence by a person covered by the licence; or
 - (c) becomes aware of any unintended effects of the dealings authorised by the licence.

Note: This condition mirrors the statutory condition in section 65 of the Act.

People dealing with GMO must allow auditing and monitoring of the dealing

10. If a person is authorised by this licence to deal with a GMO and a particular condition of this licence applies to the dealing by that person, the person must allow the Regulator, or a person authorised by the Regulator, to enter premises where the dealing is being undertaken, for the purposes of auditing or monitoring the dealing.

Note: This condition mirrors the statutory condition in section 64 of the Act.

Remaining an Accredited organisation and appointment of Project Supervisor

- 11. The licence holder must, at all times, remain an accredited organisation in accordance with the Act and comply with any conditions of accreditation set out in the Regulator's Guidelines for Accreditation of Organisations.
- 12. The licence holder must appoint a Project Supervisor to act as a point of liaison between the OGTR and the licence holder on all matters in connection with the administration of the licence.
- 13. The licence holder must immediately notify the Regulator in writing if any of the contact details of the Project Supervisor change.
- 14. The licence holder may change the Project Supervisor by notice in writing to the Regulator.

PART 3 SPECIFIC CONDITIONS

Testing Methodology

15. The licence holder must provide a written instrument to the Regulator describing an experimental method that is capable of reliably detecting the presence of the GMOs covered by this licence and any transferred genetically modified material that might be present in a recipient organism. The instrument must be provided within 30 days of this licence being issued.

Annual Report

16. Each year, the licence holder must prepare a written annual report on the administration of the licence for the previous year.
17. The period for an annual report is the year ending on anniversary of the day this licence is issued.
18. An annual report must be provided to the Regulator within 90 days of the end of each period. An annual report must be prepared and provided in accordance with any Guidelines issued by the Regulator in relation to annual reporting.
19. An annual report must include the following:
 - (a) Information about any adverse impacts, unintended effects, or new information relating to risks, to human health and safety or the environment caused by the GMOs or material from the GMOs;
 - (b) Information about the volumes of each GMO, separate and aggregate, grown for commercial purposes, including seed increase operations, in each State and Territory for each growing season in the period;
 - (c) Information about the volumes of each of the GMOs, separate and aggregate, grown for non-commercial (eg research) purposes in each State and Territory for each growing season in the period;
 - (d) Other information on the progress of the release of the GMOs, including annual surveys, the details of which will be determined in consultation with the OGTR.

REASONS FOR LICENCE CONDITIONS

General licence conditions

Other than general condition 7, the general licence conditions in Part 2 of the licence restate the statutory licence conditions that apply to the licence. General licence condition 7 contains an additional requirement. The additional requirement is that the licence holder must provide written evidence that it has informed relevant people of licence conditions that apply to them, upon the Regulator's request. This condition has been inserted because it is considered desirable to create a paper trail that the OGTR can follow, should the need arise, to determine whether people have been informed of their obligations under the licence. A blanket requirement requiring written evidence in every situation would be impractical and overly burdensome given the nature of this licence. Accordingly, in this instance, a requirement to provide documentary evidence on request is an appropriate mechanism.

Specific licence conditions

Specific condition 1 requires the licence holder to provide a testing methodology to the Regulator that is capable of reliably detecting the presence of the GMO. The condition has been imposed because it is considered to be necessary to enable the Regulator to determine whether this licence covers a particular organism, which, in turn is necessary to facilitate the effective and efficient administration of this licence, particularly routine monitoring and auditing of dealings authorised by the licence.

Specific condition 2 requires information about the quantities of the GMOs released in Australia to be reported to the Regulator each year, and has been imposed to enable continuing oversight of the progress of the commercial release of this GM canola.

Attachment A

GENERAL INFORMATION ABOUT THE GMOs COVERED BY THIS LICENCE:

GMOs Description

Parent Organism(s) Common Name: Canola

Parent Organism(s) Scientific Name: *Brassica napus*

Modified Trait(s):

Category: Herbicide tolerance
Hybrid Breeding System

Description:

Canola has been genetically modified to:

- express a gene conferring tolerance to the herbicide glufosinate ammonium;
- introduce a novel hybrid breeding system for canola, based on genetically modified male sterile (MS) and fertility restorer (RF) lines;
- express an antibiotic resistance gene.

SPECIFIC INFORMATION ABOUT THE GMOs COVERED BY THIS LICENCE

The GMOs covered by this licence are:

- (a) InVigor[®] hybrid canola (hybrids of canola containing transformation event MS8 and canola containing transformation event RF3).
- (b) the GMOs described in the table below

Table of GMOs covered by this licence

Column	GMO	Traits	Gene for glufosinate ammonium tolerance	Gene for hybrid breeding system (InVigor [®])	Gene for antibiotic resistance
1	Canola containing transformation event T45	glufosinate ammonium tolerant	<i>pat</i>		
2	Canola containing transformation event Topas 19/2	glufosinate ammonium tolerant, antibiotic resistant	<i>pat</i>		<i>nptII</i>
3	Canola containing transformation event RF1	glufosinate ammonium tolerant, antibiotic resistant, fertility restorer	<i>bar</i>	<i>barstar</i>	<i>nptII</i>
4	Canola containing transformation event RF2	glufosinate ammonium tolerant, antibiotic resistant, fertility restorer	<i>bar</i>	<i>barstar</i>	<i>nptII</i>

5	Canola containing transformation event RF3	glufosinate ammonium tolerant, fertility restorer	<i>bar</i>	<i>barstar</i>	
6	Canola containing transformation event MS1	glufosinate ammonium tolerant, male sterile, antibiotic resistant	<i>bar</i>	<i>barnase</i>	<i>nptII</i>
7	Canola containing transformation event MS8	glufosinate ammonium tolerant, male sterile	<i>bar</i>	<i>barnase</i>	

Note: the transformation events are described in the Risk Assessment and Risk Management Plan prepared in connection with this licence.

APPENDIX 9 LEGISLATIVE REQUIREMENTS FOR ASSESSING DEALINGS INVOLVING INTENTIONAL RELEASES

SECTION 1 THE REGULATION OF GENE TECHNOLOGY IN AUSTRALIA

664. The Gene Technology Act 2000 (the Act) took effect on 21 June 2001. The Act, supported by the Gene Technology Regulations 2001 (the Regulations), an inter-governmental agreement, and corresponding legislation that is being enacted in each State and Territory, underpins Australia's nationally consistent regulatory system for gene technology. Its objective is to protect the health and safety of people, and the environment, by identifying risks posed by or as a result of gene technology, and managing those risks by regulating certain dealings with genetically modified organisms (GMOs). The regulatory system replaces the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC).
665. The Act establishes a statutory officer, the Gene Technology Regulator (the Regulator), to administer the legislation and make decisions under the legislation.
666. The Regulator is supported by the Office of the Gene Technology Regulator (OGTR), a Commonwealth regulatory agency located within the Health and Ageing portfolio.
667. The Act prohibits persons from dealing with GMOs unless the dealing is exempt, a Notifiable Low Risk Dealing, on the Register of GMOs, or licensed by the Regulator (see Section 31 of the Act).
668. The requirements under the legislation for consultation and for considering and assessing licence applications and preparing risk assessment and risk management plans are discussed in detail in Division 4, Part 5 of the Act and summarised below.
669. Detailed information about the national regulatory system and the gene technology legislation is also available from the OGTR website (www.ogtr.gov.au)

SECTION 2 THE LICENCE APPLICATION

670. Applications for a DIR licence must be submitted in accordance with the requirements of Section 40 of the Act. As required by Schedule 4, Part 2 of the Regulations, the application must include information about:
- the parent organism;
 - the GMOs;
 - the proposed dealing with the GMOs;
 - interaction between the GMOs and the environment;
 - risks the GMOs may pose to the health and safety of people;
 - risk management;
 - previous assessments of approvals; and
 - the suitability of the applicant.
671. The application must also contain supporting information from the Institutional Biosafety Committee and additional information required for a GMO that is:
- a plant;
 - a micro-organism (not living in or on animals and not a live vaccine);
 - a micro-organism that lives in or on animals;
 - a live vaccine for use in animals;
 - a vertebrate animal;

- an aquatic organism;
 - an invertebrate animal;
 - to be used for biological control;
 - to be used for bioremediation; and
 - intended to be used as food for human or vertebrate animal consumption.
672. A preliminary screening of an application is undertaken by OGTR staff to determine whether it complies with the Act and the Regulations, by containing the required information. If this information is provided in the application, the Regulator may then accept the application for formal consideration. Section 43 of the Act provides that the Regulator is not required to consider an application if the application does not contain the required information.
673. After accepting an application for consideration, the Regulator must decide to issue, or refuse to issue, a licence. The decision must be taken following an extensive consultation and evaluation process, as detailed in Sections 3-6 of this Appendix. Regulation 8 of the Regulations prescribe a period of 170 working days within which this decision must be taken. This period does not include weekends or public holidays in the Australian Capital Territory. Also, this period does not include any days in which the Regulator is unable to progress the application because information sought from the applicant in relation to the application has not been received.

SECTION 3 THE INITIAL CONSULTATION PROCESSES

674. In accordance with Section 50 of the Act, the Regulator must seek advice in preparing a risk assessment and risk management plan (RARMP) from prescribed agencies:
- State and Territory Governments;
 - the Gene Technology Technical Advisory Committee (GTTAC);
 - prescribed Commonwealth agencies (Regulation 9 of the *Gene Technology Regulations 2001* refers);
 - the Environment Minister; and
 - relevant local council(s) where the release is proposed.
675. Section 49 of the Act requires that if the Regulator is satisfied that at least one of the dealings proposed to be authorised by the licence may pose significant risks to the health and safety of people or to the environment, the Regulator must publish a notice in respect of the application inviting written submissions on whether the licence should be issued.
676. As a measure over and above those required under the Act, in order to promote the openness and transparency of the regulatory system, the Regulator may take other steps. For example, receipt of applications is notified to the public by posting a notice of each application's receipt on the OGTR website and directly advising those on the OGTR mailing list. A copy of applications is available on request from the OGTR.

SECTION 4 THE EVALUATION PROCESSES

677. The risk assessment process is carried out in accordance with the Act and the Regulations, using the Risk Analysis Framework (the Framework) developed by the Regulator (available on the OGTR website). It also takes into account the guidelines and risk assessment strategies used by related agencies both in Australia and overseas. The Framework was developed in consultation with the States and Territories,

Commonwealth government agencies, GTTAC and the public. Its purpose is to provide general guidance to applicants and evaluators and other stakeholders in identifying and assessing the risks posed by GMOs and in determining the measures necessary to manage any such risks.

678. In undertaking a risk assessment, the following are considered and analysed:
- the data presented in the proponent's application;
 - data provided previously to GMAC, the interim OGTR, or the OGTR in respect of previous releases of relevant GMOs;
 - submissions or advice from States and Territories, Commonwealth agencies and the Environment Minister, and the public;
 - advice from GTTAC;
 - information from other national regulatory agencies; and
 - current scientific knowledge and the scientific literature.
679. In considering this information and preparing the risk assessment and risk management plan, the following specific matters are taken into account, as set out in Section 49 and required by Section 51 of the Act:
- the risks posed to human health and safety or risks to the environment;
 - the properties of the organism to which the dealings relate before it became, or will become, a GMO;
 - the effect, or the expected effect, of the genetic modification that has occurred, or will occur, on the properties of the organism;
 - provisions for limiting the dissemination or persistence of the GMO or its genetic material in the environment;
 - the potential for spread or persistence of the GMO or its genetic material in the environment;
 - the extent or scale of the proposed dealings;
 - any likely impacts of the proposed dealings on the health and safety of people.
680. In accordance with Regulation 10 of the Regulations, the following are also taken into account:
- any previous assessment, in Australia or overseas, in relation to allowing or approving dealings with the GMO;
 - the potential of the GMO concerned to:
 - be harmful to other organisms;
 - adversely affect any ecosystems;
 - transfer genetic material to another organism;
 - spread, or persist, in the environment;
 - have, in comparison to related organisms, a selective advantage in the environment; and
 - be toxic, allergenic or pathogenic to other organisms.
 - the short and long term when taking these factors into account.

SECTION 5 FURTHER CONSULTATION

681. Having prepared a RARMP the Regulator must, under Section 52 of the Act, seek comment from stakeholders, including those outlined in Section 3 and the public.
682. All issues relating to the protection of human health and safety and the environment raised in written submissions on an application or RARMP are considered carefully, and weighed against the body of current scientific information, in reaching the conclusions set out in a final RARMP. Section 56 of the Act requires that these be

taken into account in making a decision on whether or not to issue a licence for the proposed release.

683. Comments received in written submissions on this risk assessment and risk management plan are very important in shaping the final risk assessment and risk management plan and in informing the Regulator's final decision on an application. A summary of public submissions and an indication of where such issues have been taken into account are provided in an Appendix to the final risk assessment and risk management plan.
684. It is important to note that the legislation requires the Regulator to base the licence decision on whether risks posed by the dealings are able to be managed so as to protect human health and safety and the environment. Matters in submissions that do not address these issues and/or concern broader issues outside the objective of the legislation will not be considered in the assessment process. In most instances, as determined in the extensive consultation process that led to the development of the legislation, they fall within the responsibilities of other authorities.

SECTION 6 DECISION ON LICENCE

685. Having taken the required steps for assessment of a licence application, the Regulator must decide whether to issue or refuse a licence (Section 55 of the Act). The Regulator must not issue the licence unless the Regulator is satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in such a way as to protect the health and safety of people and the environment.
686. The Regulator must also be satisfied, under section 57 of the Act, that the applicant is a suitable person to hold the licence. Section 58 outlines matters the Regulator must consider in deciding whether a person or company is suitable to hold a licence eg.:
- any relevant convictions;
 - any relevant revocations or suspensions of a licences or permits; and
 - the capacity of the person or company to meet the conditions of the licence.
687. The Regulator carefully considers all of this information which is supplied in a declaration signed by licence applicants.
688. The Monitoring and Compliance Section of the OGTR compiles compliance histories of applicants, considering all previous approvals to deal with GMOs under the Act and the previous voluntary system. These histories as well as other information such as follow-up actions from audits may be taken into account. The ability of an organisation to provide resources to adequately meet monitoring and compliance requirements may also be taken into account.
689. If a licence is issued, the Regulator may impose licence conditions (Section 62 of the Act). For example, conditions may be imposed to:
- limit the scope of the dealings;
 - require documentation and record-keeping;
 - require a level of containment;
 - specify waste disposal methods;
 - manage risks posed to the health and safety of people, or to the environment;
 - require data collection, including studies to be conducted;
 - limit the geographic area in which the dealings may occur;
 - require contingency planning in respect of unintended effects of the dealings; and

- limiting the dissemination or persistence of the GMO or its genetic material in the environment.
690. It is also required as a condition of a licence that the licence holder inform any person covered by the licence of any condition of the licence which applies to them (Section 63). Access to the site of a dealing must also be provided to persons authorised by the regulator for the purpose of auditing and monitoring the dealing and compliance with other licence conditions (Section 64). It is a condition of any licence that the licence holder inform the Regulator of:
- any new information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence;
 - any contraventions of the licence by a person covered by the licence; and
 - any unintended effects of the dealings authorised by the licence.
691. If the Regulator decides to issue a licence, she will continue to proactively review any new information about risks of the proposed release and may amend or add licence conditions accordingly. Under section 68 of the Act, the Regulator may also suspend or cancel a licence if a licence has been breached or if the Regulator becomes aware of new risks that are not adequately managed. The Regulator can also vary a licence to impose extra management conditions if necessary.
692. It should be noted that, as well as imposing licence conditions, the Regulator has additional options for risk management. The Regulator has the legislative capacity to enforce compliance with licence conditions, and indeed, to direct a licence holder to take any steps the Regulator deems necessary to protect the health and safety of people or the environment. The OGTR also independently monitors trial sites to determine whether the licence holder is complying with the licence conditions, or whether there are any unforeseen problems.

APPENDIX 10 SUMMARY OF PUBLIC SUBMISSIONS ON THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN

OVERVIEW

693. The OGTR received 256 written submissions from individuals and organisations during the public consultation process on the RARMP.
694. A total of 531 ‘campaign’ letters and e-mails (eight types in all were received) and five (5) petitions were also received, representing 471 signatories. Those that expressed positions against GMOs in general, or the proposed release in particular, without raising risks to human health and safety or the environment could not be taken into account in the assessment process.
695. A total of eleven (11) types of issue were raised which can be categorised into three broad groups:
- issues within the scope of the Gene Technology Act 2000;
 - issues which are the responsibility of other agencies; and
 - issues which are outside the scope of assessments under the Act

DETAILED CONSIDERATION OF ISSUES

696. The accompanying table at the end of this appendix analyses the issues raised in the public submissions in detail. The first column notes the type of organisation that made the submission and the remaining column headers indicate which of the eleven (11) issues were raised.

Issues within the scope of the Act

697. This includes matters related to the protection of human health and safety and the environment and also the suitability of Bayer CropScience Pty Ltd to hold a licence in accordance with section 58 of the Act.
698. While all issues raised relating to risks to human health and safety and/or the environment were addressed in the consultation version of the RARMP, the consultation process highlighted particular areas of concern, and in some instances confusion. Therefore, (as outlined in Chapter 2 Section 1) relevant areas of the final plan have been considerably revised and expanded to further explain the evaluation process and the basis of the conclusions reached as follows:
- 699.

Issue	Enhanced explanation
1. General Health concerns	see Appendix 2
2. Precaution and general safety	see Appendix 8 and Appendix 4 Section 1
3. General environmental concerns	see Appendices 3, 4 and 5
4. Pollen flow and “contamination	see Appendix 5
5. Herbicide resistant weeds	see Appendix 4
6. Applicant suitability	see Chapter 2 Section 4, Appendix 8 Section 6

Issues which are the responsibility of other agencies

700. Many submissions raised issues that related to matters that are the responsibility of either Agricultural Pesticides and Veterinary Medicines Authority (formerly the National Registration Authority, NRA), which is responsible for regulating the safety and use of herbicides and pesticides, and product efficacy, including resistance management strategies; or Food Standards Australia New Zealand (FSANZ, formerly the Australia New Zealand Food Authority), which is responsible for food safety and labelling, including GM foods.

701. This group of issues comprises the following categories :

Issue
7. Herbicide use and resistance management
8. Safety and labelling of GM foods

Outside the Scope of Assessment

702. Public submissions raised a number of issues, such as impacts on domestic and export markets, costs and adequacy of segregation protocols, liability and impacts on organic status, that are outside the scope of the evaluations conducted under the Act and therefore could not be considered as part of the assessment process.

703. Extensive consultations during the development of the Act determined that trade and economic issues such as these would be excluded from consideration by the Regulator in deciding whether to approve licences. This was to ensure that the regulatory system's scientifically-based assessment of risks to human health and safety and the environment was not compromised by consideration of economic issues.

704. As these issues are outside the scope of the assessment, the RAMP can not give them specific consideration. However, the RARMP does have some discussion of these issues in the sections indicated:

Issue	Enhanced explanation
9. Agricultural practices	see Appendices 3, 4 and 5
10. Economic/market issues	see Chapter 2 Section 2, Appendix 4 Section 2.2, Appendix 5 Section 2.2 and Appendix 7
11. Other general issues	

Organisation key:

A Submission from: A: agricultural organisation; I: individual; E: environmental organisation; F: food interest organisation; C: consumer/public interest organisation; Pe: Petition*; Ca: Campaign form letter**

Matters within the scope of the <i>Gene Technology Act 2000</i> (the Act) – columns 1-6	Matters which are the responsibility of the APVMA – column 7 and matters which are the responsibility of FSANZ – column 8	Matters outside the scope of the Act – columns 9-11
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No.	Organisation category	1 General health concerns	2 Precaution and general safety	3 General environment concerns	4 Pollen flow and 'contamination'	5 Herbicide resistant weeds	6 Applicant suitability	7 Herbicide use and resistance Management	8 Safety and labelling of GM food	9 Agricultural Practice	10 Economic/market issues	11 Other general issues
1	I		X				X					
2	I		X		X		X				X	
3	I					X						
4	I		X				X					
5	I		X		X							
6	I		X		X				X		X	
7	I		X				X					
8	I	X	X			X			X			
9	I	X			X				X		X	
10	I	X							X			
11	I		X		X	X						
12	I			X	X		X					
13	I						X		X		X	
14	I			X							X	
15	I	X										
16	I				X						X	
17	I											X
18	I				X						X	
19	I	X	X						X			
20	I				X						X	
21	I	X							X		X	
22	I	X					X		X		X	
23	I											X
24	I											X
25	I											X
26	I				X	X						
27	I				X							
28	I	X	X	X	X		X		X			
29	I	X			X				X			
30	I											X
31	I				X						X	
32	I			X	X						X	
33	I										X	
34	I				X						X	
35	I			X		X				X		
36	I		X		X	X	X					
37	I	X	X		X		X		X			
38	I	X	X								X	
39	I	X	X				X		X			
40	Ca	X	X		X	X		X	X		X	
41	Ca	X	X		X				X			
42	I	X	X	X					X	X		
43	I					X					X	
44	I								X		X	
45	C											X
46	A	X	X		X						X	
47	I	X	X		X		X				X	
48	I				X							
49	I		X									
50	I											X
51	I	X			X				X			
52	I										X	X
53	I		X		X				X			

No.	Organisation category	1 General health concerns	2 Precaution and general safety	3 General environment concerns	4 Pollen flow and 'contamination'	5 Herbicide resistant weeds	6 Applicant suitability	7 Herbicide use and resistance Management	8 Safety and labelling of GM food	9 Agricultural Practice	10 Economic/ market issues	11 Other general issues
54	I		X									
55	I						X					
56	I		X				X					
57	I			X						X		
58	I		X					X				
59	I											X
60	C	X	X	X	X		X	X			X	
61	I	X	X	X	X	X	X	X			X	
62	C											X
63	I	X	X		X	X						
64	I	X	X		X							
65	F	X	X	X	X	X	X	X			X	
66	I											X
67	I											X
68	I				X	X						
69	I											X
70	I		X									
71	I	X		X	X		X				X	
72	I	X		X	X	X		X				
73	I				X							
74	I							X				
75	C											X
76	Ca	X	X	X	X	X	X	X			X	
77	I				X		X					
78	Pe				X	X	X	X			X	
79	I	X	X		X	X	X					
80	A				X	X					X	
81	A	X	X		X	X	X					
82	I			X		X					X	
83	A											X
84	I		X	X	X	X						
85	A											X
86	I											X
87	C		X									
88	I							X				
89	I							X				
90	C	X	X					X			X	
91	I	X	X				X					
92	I			X	X	X						
93	I	X	X					X				
94	I		X		X							
95	I											
96	I											X
97	I						X					
98	I											X
99	I	X	X		X	X	X	X			X	
100	I						X				X	
101	I											X
102	I	X		X	X	X		X			X	
103	I	X						X				
104	I				X			X			X	
105	I											X
106	I	X	X		X	X	X	X				
107	I							X				
108	I											X
109	I				X	X	X	X				
110	I										X	
111	I				X			X				
112	I	X	X									
113	I											X
114	I	X	X	X						X		
115	I	X	X		X			X				
116	I	X	X		X		X				X	
117	I		X		X		X					
118	I											X

No.	Organisation category	1 General health concerns	2 Precaution and general safety	3 General environment concerns	4 Pollen flow and 'contamination'	5 Herbicide resistant weeds	6 Applicant suitability	7 Herbicide use and resistance Management	8 Safety and labelling of GM food	9 Agricultural Practice	10 Economic/ market issues	11 Other general issues
119	I											X
120	I	X					X					
121	I				X		X				X	
122	I	X	X	X								
123	I											X
124	I		X	X	X	X	X				X	
125	I						X				X	
126	I			X			X	X			X	
127	I		X		X	X	X				X	
128	I	X					X	X				
129	I						X				X	
130	E		X	X	X		X					
131	I		X				X				X	
132	C											X
133	I											X
134	I		X									
135	I	X									X	
136	I				X		X				X	
137	I	X	X	X	X						X	
138	I		X				X					
139	I		X				X	X				
140	I											X
141	I		X	X		X						
142	I											X
143	I											X
144	I				X							
145	E			X	X		X					
146	I											X
147	I											X
148	I		X									
149	I	X		X	X		X	X			X	
150	I											X
151	I				X	X	X			X	X	
152	I											X
153	A											X
154	A		X		X	X						
155	I											X
156	A											X
157	I	X	X	X	X			X				
158	C	X	X	X	X		X	X			X	
159	I	X			X		X				X	
160	I											X
161	C		X	X				X			X	
162	I		X	X			X					
163	I											X
164	I	X			X			X				
165	I											X
166	I	X						X			X	
167	I			X	X	X					X	
168	I			X	X		X				X	
169	C		X		X							
170	C	X	X	X	X	X		X	X	X		
171	I											X
172	E											X
173	I											X
174	C											X
175	C	X	X				X	X				
176	E		X									
177	Pe											X
178	I			X	X						X	
179	C	X		X	X	X						
180	C	X	X	X			X					
181	I	X	X	X			X					
182	I						X					
183	I	X	X	X	X	X		X	X	X		

No.	Organisation category	1 General health concerns	2 Precaution and general safety	3 General environment concerns	4 Pollen flow and 'contamination'	5 Herbicide resistant weeds	6 Applicant suitability	7 Herbicide use and resistance Management	8 Safety and labelling of GM food	9 Agricultural Practice	10 Economic/ market issues	11 Other general issues
184	I		X								X	
185	C	X	X				X				X	
186	C	X		X	X				X	X		
187	I		X					X			X	
188	I	X					X					
189	I	X		X	X		X				X	
190	I			X	X	X						
191	I		X					X				
192	I		X		X							
193	I	X	X		X			X				
194	I	X	X		X	X					X	
195	I	X					X					
196	I			X			X				X	
197	I	X		X			X	X				
198	I			X	X	X	X				X	
199	I		X				X	X				
200	I				X		X					
201	I		X									
202	I							X				
203	I		X		X						X	
204	I		X					X				
205	I			X	X		X					
206	I				X						X	
207	I											X
208	I		X				X				X	
209	I		X				X					
210	I						X	X			X	
211	I						X					
212	I	X	X	X	X		X					
213	I						X					
214	I											X
215	I				X							
216	I				X	X					X	
217	I	X	X	X			X					
218	I		X		X		X				X	
219	I						X	X				
220	I		X				X					
221	I	X	X				X	X				
222	I	X		X	X	X		X			X	
223	I		X				X					
224	I						X	X				
225	I		X	X	X							
226	I	X		X				X			X	
227	I		X									
228	I	X			X		X				X	
229	I	X	X	X								
230	I			X	X	X						
231	I	X	X		X		X					
232	I			X								
233	I				X							
234	I				X							
235	I		X	X			X					
236	E		X								X	
237	I	X	X	X	X	X	X				X	
238	I											
239	I	X	X		X						X	
240	I	X	X				X					
241	I		X		X						X	
242	I	X					X					
243	I			X			X				X	
244	I			X			X				X	
245	Pe											X
246	C	X	X	X	X		X	X			X	X
247	A											X
248	A											X

No.	Organisation category	1 General health concerns	2 Precaution and general safety	3 General environment concerns	4 Pollen flow and 'contamination'	5 Herbicide resistant weeds	6 Applicant suitability	7 Herbicide use and resistance Management	8 Safety and labelling of GM food	9 Agricultural Practice	10 Economic/ market issues	11 Other general issues
249	E	X	X	X	X	X			X		X	
250	Ca	X		X	X						X	
251	Ca		X						X			
252	Ca	X		X	X	X			X			
253	Ca	X	X	X			X		X		X	
254	Ca	X	X	X	X		X				X	
255	Pe								X			
256	Pe	X	X	X		X						

*, ** A number of 'campaign' letters and petitions were received as follows:

Campaign letter 1	462
Campaign letter 2	4
Campaign letter 3	17
Campaign letter 4	13
Campaign letter 5	7
Campaign letter 6	3
Campaign letter 7	5
Campaign letter 8	20

Petition 1	236 Signatures
Petition 2	76 Signatures
Petition 3	70 Signatures
Petition 4	75 Signatures
Petition 5	14 Signatures

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