Risk Assessment and Risk Management Plan

Final Version

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

DIR 020/2002

Title: **General Release of Roundup Ready® canola (*Brassica napus*) in Australia**

Applicant: Monsanto Australia Ltd

December 2003

Abbreviations and Definitions

a.i.	active ingredient
aad	gene conferring resistance to bacteria to streptomycin and spectinomycin antibiotics. Located in *Agrobacterium* binary vector but not transferred to Roundup Ready® canola
AAFC	Agriculture and Agri-Food Canada
AFLP	amplified fragment-length polymorphism
AMPA	aminomethylphosphonic acid, breakdown product of glyphosate
ANZFA	Australia New Zealand Food Authority
APHIS	Animal and Plant Health Inspection Service
APVMA	Australian Pesticides and Veterinary Medicines Authority (formerly NRA)
BC	back cross
binary vector	plasmid that carries the genes to be inserted into the plant. Developed from the Ti plasmid of *Agrobacterium* by deleting the tumour inducing genes within the T-DNA
BLAST	Basic Local Alignment Search Tool
CaMV	Cauliflower Mosaic Virus
CLPP	community level physiological profiling
CONABIA	Comisión Nacional Asesora de Biotecnología Agropecuaria (National Advisory Commission on Agricultural Biotechnology) in Argentina
CP4 EPSPS	5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium* sp. CP4
CP4 EPSPS	5-enolpyruvylshikimate-3-phosphate synthase enzyme from *Agrobacterium* sp. CP4, that is tolerant to glyphosate
CTP1	chloroplast transit peptide sequence derived from the *rbcS* gene of *Arabidopsis thaliana*
CTP2	chloroplast transit peptide sequence from the *epsps* gene of *Arabidopsis thaliana*
DDBJ	DNA Databank of Japan
DEFRA	The Department of Environment, Food and Rural Affairs, UK
DIR	dealing involving intentional release
DNA	deoxyribonucleic acid
E9 3'	Signals at end of introduced genes, from the E9 Rubisco gene of pea
ELISA	enzyme linked immunosorbent assay
EMBL	European Molecular Biology Laboratory
FAME	fatty acid methyl ester profile
FAO	Food and Agriculture Organisation of the United Nations
FASTA	computer program used to compare a DNA or protein sequence to a database of DNA or protein sequences
FDA	Food and Drug Administration (USA)
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act (USA)
FMV	Figwort Mosaic Virus
FSANZ	Food Standards Australia New Zealand
g	gram
GM	genetically modified
GMAC	Genetic Manipulation Advisory Committee
GMO	genetically modified organism
goxv247	glyphosate oxidoreductase variant 247 gene
GOXv247	glyphosate oxidoreductase enzyme
GT73	Roundup Ready® canola line
GTGC	Gene Technology Grains Committee
GTTAC	Gene Technology Technical Advisory Committee
ha	hectare
iaaH	gene from wild-type *Agrobacterium tumefaciens* that allows the bacteria to cause crown-gall disease
iaaM	gene from wild-type *Agrobacterium tumefaciens* that allows the bacteria to cause crown-gall disease
IgE	immunoglobulin E
IOGTR	Interim Office of the Gene Technology Regulator
ipt	gene from wild-type *Agrobacterium tumefaciens* that allows the bacteria to cause crown-gall disease
kD	kiloDaltons
km	kilometre

m	metre
MAFF	UK Ministry of Agriculture, Fisheries and Food (now called DEFRA)
mg	milligrams
mRNA	messenger ribonucleic acid
nos	derived from the nopaline synthase gene of *Agrobacterium* sp.
NPTII	neomycin phosphotransferase II enzyme
NRA	National Registration Authority for Agricultural and Veterinary Chemicals (now APVMA)
OECD	Organisation for Economic Cooperation and Development
OGTR	Office of the Gene Technology Regulator
ORF	open reading frame
P-CMoVb	promoter from figwort mosaic virus used to drive the introduced genes
PCR	polymerase chain reaction
PDB	The Protein Data Bank
ppm	parts per million
PR	planned release
PV-BNGT04	a binary vector of Agrobacterium that carried the genes to be inserted into Roundup Ready® canola
R0, R1, R3..R5	generations of canola since transformation
RARMP	Risk Assessment and Risk Management Plan
RNA	ribonucleic acid
RRCMP	Roundup Ready® canola Crop Management Plan
RRRMP	Roundup Ready® canola Resistance Management Plan
RRTM	Roundup Ready® canola Technical Manual
RRTUA	Roundup Ready® canola Technology User Agreement
Rubisco	ribulose-1,5-bisphosphate carboxylase enzyme
SGF	simulated gastric fluid
SIF	simulated intestinal fluid
Spc^R	designates a bacteria carrying spectinomycin resistance
Str^R	designates a bacteria carrying streptomycin resistance
T-DNA	transfer deoxyribonucleic acid of *Agrobacterium.* Delineated by the Left and Right border sequences
Ti plasmid	'Tumour inducing' plasmid of *Agrobacterium*. This plasmid has been replaced by a binary vector in 'disarmed' *Agrobacterium* strains used for plant transformation.
Tn7	segment of DNA from the bacterium *Escherichia coli*
UK	United Kingdom
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
vir	virulence genes of *Agrobacterium*
WHO	World Health Organisation
µg	micrograms
µm	micromoles

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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 cannot 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 stakeholders including the public. The RARMP forms the basis of her decision whether or not to issue a licence.

The Act is designed to operate in a cooperative legislative framework with other regulatory authorities that have complementary responsibilities and specialist expertise. As well as enhancing coordinated decision making, this arrangement avoids duplication.

The Gene Technology Regulator is responsible for the evaluation of all applications for contained research and early stage trial work with GMOs in Australia. However, once a GMO reaches later stage development or commercial application, other product approval authorities also have a role. For example Food Standards Australia New Zealand (FSANZ) sets the standards for safety and labelling of foods for human consumption. Approvals may be sought for imported GM foodstuffs, prior to seeking approval from the Regulator to grow the crop in Australia.

Similarly, the Agricultural Pesticides and Veterinary Medicines Authority (APVMA) is responsible for assessing the safety and ensuring the efficacy of all agricultural chemicals and veterinary medicines on a whole of sector basis. Insecticidal GM crops must be registered by the APVMA as well as licensed for release by to the environment by the Regulator, and the use of registered herbicides on GMOs (such as Roundup Ready® herbicide on Roundup Ready® canola) normally requires the approval of an extension of use to the registration.

The Regulator is required to seek input from both FSANZ and the APVMA during the preparation of the RARMP, as well and the Therapeutic Goods Administration which regulate pharmaceuticals and the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) which regulates the use of industrial chemicals.

All of these agencies are required to advise the Regulator if they approve a product that is, or was produced by, a GMO in order for her to maintain a public record, on the OGTR website of all dealings undertaken with GMOs in Australia.

THE APPLICATION

In June 2002, Monsanto Australia Ltd (Monsanto) applied for a licence (application number DIR 020/2002) for the commercial release of genetically modified (GM) canola (*Brassica napus*) into the environment.

The GM canola that Monsanto sought approval for is Roundup Ready® canola derived from transformation event GT73. Roundup Ready® canola is tolerant to the herbicide glyphosate.

Glyphosate is a broad-spectrum herbicide and is the active constituent of a range of proprietary herbicides, including Roundup®, registered by the Australian Pesticides and Veterinary Medicines Authority (APVMA). Glyphosate has been registered for use in non-selective (general) weed control in broadacre agriculture, horticulture and non-cropped areas including industrial areas and roadsides and is a widely used chemical in all these situations.

Conventional canola is sensitive to glyphosate, so the herbicide cannot be used for weed control in canola crops. Glyphosate can however applied to Roundup Ready® canola without killing it, because of the introduced herbicide tolerance genes.

Glyphosate is registered under the trade name 'Roundup Ready®' herbicide by Monsanto' for use on Roundup Ready® cotton in Australia, but has not previously been registered for use on Roundup Ready® canola.

The APVMA has recently approved an extension of use on the registration of Roundup Ready® herbicide to enable its application 'over the top' of Roundup Ready® canola crops to control post-emergent weeds (*ie.* once the crop has been planted and germinated). Appendices 4 and 6 of the RARMP contain further details.

Monsanto's application to the Gene Technology Regulator proposed commercial cultivation of Roundup Ready® canola in all current and future canola growing regions of Australia without specifying any containment measures.

Subject to approval, Monsanto anticipated a steady increase in the area sown to Roundup Ready® canola over a number of years across the canola growing regions of Australia, with the rate of increase being determined by market acceptance, State Government agreement and seed and variety availability.

Monsanto stated its intention to continue to work closely with the grains industry and State and Territory Governments to manage the introduction of Roundup Ready® canola.

Roundup Ready® canola from this release is intended for use as oil in human food, or in animal feed, in the same way as conventional (non-GM) canola. Roundup Ready® canola has been approved for growing and human consumption in Japan, Canada and the USA. It is approved for food use in Europe and an application is pending for environmental release. Roundup Ready® canola has been trialed previously in Australia under limited and controlled conditions, and oil derived from Roundup Ready® canola has been approved by Food Standards Australia New Zealand (FSANZ) for use in human food in Australia. Chapter 1 of the RARMP provides further details.

Roundup Ready® canola has been genetically modified to be tolerant to the herbicide glyphosate by the introduction of two genes, the *CP4 EPSPS* gene from the bacterium *Agrobacterium* sp. strain CP4 and the *goxv247* gene from the bacterium *Ochrobactrum anthropi*. The *CP4 EPSPS* gene encodes the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and the *goxv247* gene encodes the enzyme glyphosate oxidoreductase (GOX).

Glyphosate kills plants by inhibiting the endogenous plant EPSPS enzyme that is involved in an important biochemical pathway for synthesis of aromatic amino acids. The pathway is not present in mammalian, avian or aquatic animals which explains the herbicide's selective action on plants. The enzyme produced by the *CP4 EPSPS* gene has a higher tolerance to the action of glyphosate than the plant's equivalent protein. Roundup Ready® canola is tolerant to glyphosate because the GOX enzyme detoxifies the glyphosate herbicide and the *CP4 EPSPS* gene has a high tolerance to glyphosate.

Under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC), Monsanto conducted five limited and controlled releases (PR77 and extensions) of Roundup Ready® canola in Queensland, New South Wales, Victoria, Tasmania, South Australia and Western Australia.

In addition, the Regulator has previously assessed an application for the limited and controlled release of Roundup Ready® canola, and authorised further field trials in Victoria, South Australia, New South Wales and Western Australia under Licence No. DIR 011/2001.

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

Some information supplied by Monsanto in response to requests by the Regulator has been declared 'Confidential Commercial Information' under section 185 of the Act. In accordance with section 184 of the Act this information was not available to the general public. However it *was* available to the expert groups and authorities that are required to be consulted on the preparation of the RARMP and the APVMA.

The information included detailed technical information on molecular characterisation of Roundup Ready® canola, and draft versions of documents relating to Monsanto's stewardship strategy for Roundup Ready® canola.

The draft documents could not be completed until regulatory approvals were received from the Regulator and the APVMA, and the final licence and registration conditions known and incorporated. Following approval by the Australian Pesticides and Veterinary Medicines Authority, many of these documents *eg.* Roundup Ready® canola Crop Management Plan, Resistance Management Plan and Tech Topics technical notes, will be available from Monsanto website or by contacting Monsanto directly.

THE RISK ASSESSMENT AND EVALUATION PROCESS

Licence application DIR 020/2002 from Monsanto was evaluated and a RARMP was prepared, in accordance with the Act and the Regulations, using the Risk Analysis Framework. This framework was developed as part of the establishment of the new regulatory arrangements in consultation with the public, key State, Territory and Australian government stakeholders, and the Gene Technology Technical Advisory Committee.

Details of the process that the Regulator must follow, including the prescribed consultation process, and the matters that must be considered in preparing a RARMP and licence, are set out in Appendix 9 of the RARMP. The complete, finalised RARMP can be obtained from the Office of the Gene Technology Regulator's (OGTR's) website or by contacting the Office on 1800 181 030.

The risk assessment considered information contained in the application (including information required by the Act and the Regulations on the GMO, the parent organism, and the proposed dealings and on potential impacts on human health and safety and the environment). The assessment also considered submissions received from expert groups and authorities consulted on the application as prescribed by the Act, invited advice from the public and the most current scientific knowledge.

As mentioned above, an extension of use to allow Roundup Ready® herbicide (a formulation of glyphosate) to be used for post emergent weed control in Roundup Ready® canola crops in Australia has been approved by the APVMA. 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, as well as herbicide efficacy and herbicide resistance management requirements.

The Gene Technology Regulator's risk assessment evaluated potential hazards that might be posed by the release of the GM canola based on the combined consideration of the likelihood of the hazard occurring and the likely impact if the hazard were realised. These hazards were considered and evaluated previously for the same GM canola under the Roundup Ready® canola field trial application DIR 011/2002, but were reassessed to determine whether the proposed scale of the release posed any additional risks.

Through this process, potential hazards to human health and safety or the environment that may be posed by the release of the GM Roundup Ready® canola were investigated. They were evaluated on the basis of the likelihood of the hazard occurring and the likely impact of the hazard, if it were to be realised. The identified potential hazards relate to:

- **Toxicity or allergenicity to humans:** could Roundup Ready® canola be more toxic or allergenic than conventional canola, as a result of the novel gene products or because of unintended effects ?
- **Toxicity to other organisms:** could Roundup Ready® canola be harmful to other organisms including mammals (other than humans), livestock, wildlife, insects and microorganisms as a result of the novel gene products or because of unintended effects ?
- **Weediness:** could Roundup Ready® canola be harmful to the environment because of inherent weediness or increased potential for weediness ?
- **Gene transfer:** could the new genes introduced into Roundup Ready® canola transfer to conventional canola crops, closely related *Brassica* weeds, related brassicaceous weeds or other organisms, with any adverse consequences for the environment ?
- **Herbicide Resistance:** as glyphosate is a widely used herbicide in Australia, both in agricultural and other situations, could weeds develop resistance to herbicide if the Roundup Ready® canola crop-herbicide combination is used inappropriately?
- **Change in herbicide use patterns:** what is the impact of using herbicides other than glyphosate to control Roundup Ready® canola volunteers ?

The consultation version of the RARMP was released for public comment on 2 October 2003. Although the Act specifies a minimum consultation of 30 days, the Regulator extended the period to eight weeks *ie.* until 28 November 2003.

Public consultation is an essential component of Australia's gene technology regulatory scheme that helps ensure that issues can be raised, hazards identified and risks investigated to determine whether or not they can be managed. Input from the public, interested organisations and government agencies on this application has provided particularly valuable feedback. The issues raised are discussed further in Chapter 2 and Appendix 10.

Comments on the RARMP for Roundup Ready® canola were wide ranging – from philosophical objections to gene technology generally, through to support for this canola variety in particular. Submissions ranged in length and substance from short one-sentence comments through to detailed papers covering many pages.

All of these submissions were analysed by OGTR. Many of the issues raised had been considered during the development of the consultation version of the RARMP. However, the consultation comments highlighted areas that required further explanation and we have sought to do this as part of this licence decision package.

Economic, trade and marketing considerations

There has been considerable speculation in the media and other forums, as well as in some submissions from the public, about the possible impact of the uptake of GM canola on conventional agriculture and upon Australia's 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 evaluation of risks to human health and safety and the environment. This was to prevent the possibility of economic considerations, such as cost-benefit analyses, market access and agricultural trade implications, from 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 Monsanto's Roundup Ready® canola to individual farmers, or on market impacts for the agricultural industry.

However, the Regulator and other government agencies are aware of the level of concern about, and the need for information on, marketing issues in particular. A number of submissions expressed disappointment that the Regulator could not consider potential economic and marketing impacts. It was therefore considered appropriate to highlight a number of government and industry initiatives (independent of this assessment) which do focus on the assessment of economic and marketability considerations in relation to the adoption of GM canola by the Australian agriculture industry.

Available documentation such issues includes:

- the ABARE report *Market Access Issues for GM Products – Implications for Australia*
- the Australian Bureau of Agricultural & Resource Economics (ABARE) report *Australian Grains Industry 2003-GM Canola. What are its economics under Australian conditions?*
- the Productivity Commission report *Modelling Possible Impacts of GM Crops on Australian Trade*
- 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

A number of other informative papers are available from the Australian Government Department of Agriculture, Fisheries and Forestry.

Further information on industry and government initiatives is provided in Appendix 7 of the RARMP.

CONCLUSIONS OF THE RISK ASSESSMENT

Following rigorous assessment, the Regulator considers that the risks posed by the proposed commercial release of Roundup Ready® canola to human health, safety and the environment are no greater than those posed by conventional (non-GM) canola. Accordingly, the Regulator has decided to issue a licence in respect of the Monsanto application DIR 020/2002, which

contains only minimal oversight conditions. The assessment of each potential hazard identified above is summarised under a separate heading below.

Toxicity or allergenicity to humans

Roundup Ready® canola is not likely to prove more toxic or allergenic to humans than conventional canola in either food or non-food uses. Studies show that the introduced proteins are not toxic, are rapidly degraded by mammalian digestive systems and do not share significant sequence homology with known protein toxins or allergens. Feeding studies with Roundup Ready® canola seed or meal demonstrate no anti-nutritional effects of the genetic modification. The composition of Roundup Ready® canola and the level of naturally occurring toxicants do not significantly differ from conventional canola. The major metabolites of glyphosate are not toxic. In addition, the introduced proteins are expressed at low levels in the GM plants and are already commonly encountered by humans in nature. Oil from the Roundup Ready® canola, which contains no detectable levels of genetic material or protein, is the only component of the canola that will be consumed by humans and has been approved for use in food by FSANZ.

Toxicity to other organisms

Roundup Ready® canola is not likely to prove more toxic to other organisms than conventional canola. As outlined above, a number of studies, including toxicity and feeding studies in a range of organisms, have shown no increased toxicity to other organisms. Therefore the risks are considered negligible and it is not considered necessary to impose any management conditions in relation to potential toxicity to other organisms.

Weediness

The risk that Roundup Ready® canola will be more invasive or persistent than conventional (non-GM) canola in Australia is negligible.

The growth characteristics and agronomic performance of Roundup Ready® canola are within the range of conventional canola.

Canola can occur as an agricultural weed, particularly as plants (known as volunteers) that germinate after harvest from fallen seed. However, because it is a highly domesticated crop, canola does not establish or persist well in undisturbed, natural habitats.

The introduction of tolerance to the herbicide glyphosate will not provide any selective advantage over conventional canola except where glyphosate is used.

Roundup Ready® canola is only tolerant to glyphosate and its susceptibility to other herbicides is no different to conventional canola. Therefore, Roundup Ready® canola can be effectively managed and controlled using alternative herbicides and other (non-chemical) weed control practices that can be applied to conventional canola.

Glyphosate is widely used for weed control (including canola volunteers) in Australia in broad-acre agriculture, horticulture and other situations. The APVMA has approved an extension of the registration of Roundup Ready® by Monsanto for post-emergent weed control in Roundup Ready® canola crops (*ie.* once the crop has been planted and the seed has germinated).

The emergence of volunteer plants subsequent to the cultivation of a crop, and their control or removal prior to the next season's planting, is an integral part of normal agricultural practice that is not in any way restricted or peculiar to either canola or GM crops. Therefore, adoption of Roundup Ready® canola will mean that farmers will need to make choices and potentially modify their farming practices. This may result in increased complexity in implementing alternative weed management strategies, as well as other economic considerations. It will not

pose any greater risks to human health and safety or the environment than conventional canola. Therefore no risk management conditions are proposed in relation to weediness.

Gene Transfer

When analysing the risk of gene transfer, it is important to distinguish between hybridisation and introgression. Hybridisation is the crossing of two different plants of the same or different species, resulting in the production of hybrid progeny. Introgression is the incorporation of the new gene into successive generations of the hybrid population. Hybridisation only occurs in a single subsequent generation of plants whereas introgression is ongoing. Therefore introgression is more likely to pose an environmental consequence.

To other canola

In a commercial situation, outcrossing between canola varieties is inevitable, but the overall frequency of out-crossing will be very low decreasing significantly at distances of over 5-10 metres. Gene transfer to other canola is most likely in close proximity to Roundup Ready® canola.

Even if gene transfer to other canola did occur, it would pose no greater risks other than the negligible risks posed by Roundup Ready® canola itself, or require management. As explained above, transfer of the herbicide tolerance genes will not confer a selective advantage in the absence of glyphosate and will not make plants more invasive or persistent. Roundup Ready® canola is only tolerant to glyphosate and it is as susceptible to other herbicides as conventional canola, and glyphosate tolerant volunteers can be controlled with other herbicides and management practices.

The emergence of glyphosate tolerant volunteers where Roundup Ready® canola has not previously been sown will mean that farmers must make choices about methods of weed control, after considering farm practice and economic issues.

Gene transfer to other canola will not pose any greater risks to human health and safety or the environment than conventional canola.

To closely related Brassica species

The likelihood of some gene transfer from Roundup Ready® canola to the closely related weedy *Brassica* species *B. rapa* and *B. juncea* is high, but less than for the transfer to canola (*B. napus*) and decreases rapidly with distance from the crop. Because of the lower incidence of these species, especially *B. juncea*, and the reduced 'fitness' of any progeny *eg.* vigour, fertility *etc.*, the overall frequency of introgression would also be lower. Gene transfer to *B. oleracea* would be unlikely, as hybrids are not readily formed.

B. rapa, *B. juncea* and *B. oleracea* are all principally weeds of agricultural cropping or disturbed habitats, but not of undisturbed natural habitats. Glyphosate tolerant hybrids would be most likely to arise within or adjacent to Roundup Ready® canola crops, where glyphosate would not be used for weed control post-harvest because it would not control Roundup Ready® canola volunteers. In such situations, measures taken to control Roundup Ready® canola would also eliminate any glyphosate tolerant *Brassica* species.

In disturbed habitats such as roadsides, glyphosate tolerant *Brassica* species can be controlled by all other herbicide and non-chemical methods currently used to control them. Glyphosate is widely used for non-selective weed control in Australia, including the control of brassicaceous weeds. Glyphosate is not the herbicide of choice for the control of all broadleaf weeds, and therefore other herbicides are often incorporated with glyphosate (tank mixing or 'spiking') in situations where there is a mixed weed spectrum or enhanced knockdown of difficult to control weeds is required.

If gene transfer from Roundup Ready® canola *B. rapa*, *B. juncea* and *B. oleracea* did occur, it would not make them more invasive or persistent. While transfer of the glyphosate tolerance trait to related species would not result in an adverse impact on the environment, it would have implications for the choice of herbicide(s) in situations where glyphosate is the principal strategy for control of these plants.

Taking into account the relative weediness, persistence and distribution of the related *Brassica* species, the risk of gene transfer from Roundup Ready® canola in a commercial situation resulting in adverse environmental impacts is considered to be very low for *B. rapa* and negligible for *B. juncea* and *B. oleracea.*

To sexually compatible brassicaceous weeds

Gene transfer from Roundup Ready® canola to the less closely related brassicaceous weed species would be restricted to *Raphanus raphanistrum, Hirschfeldia incana* and *Sinapis arvensis*. The overall frequency of outcrossing is expected to be extremely low, and the likelihood of introgression in any resulting hybrid plants is considered to be very low because and of genome incompatibility and the severely reduced 'fitness' of any progeny.

Even if gene transfer from Roundup Ready® canola to *R. raphanistrum*, *H. incana* and *S. arvensis* did occur over time, it would not make the hybrids more invasive or persistent.

Like the more closely related *Brassica* species, *R. raphanistrum*, *H. incana* and *S. arvensis* are all principally weeds of agricultural cropping or disturbed habitats, but not of undisturbed natural habitats. Glyphosate tolerant hybrids would be most likely to arise within or adjacent to Roundup Ready® canola crops where glyphosate would not be used for weed control post-harvest because it would not control Roundup Ready® canola volunteers.

Glyphosate is widely used for non-selective weed control in disturbed habitats in Australia, including the control of brassicaceous weeds. As for the related *Brassica* species, transfer of the glyphosate tolerance trait to these species would not result in an adverse impact on the environment but it would have implications for the choice of herbicide(s) in situations where glyphosate is the principal strategy for control of these plants. Glyphosate tolerant brassicaceous weeds would be effectively controlled by all other herbicide and non-chemical methods that are currently used to control them.

Taking into account the relative weediness, persistence and distribution of these species, the risk of gene transfer to any of these brassicaceous weeds in a commercial situation resulting in adverse impacts on human health and safety or the environment is considered to be very low.

To other brassicaceous species

Natural hybridisation between canola and other brassicaceous species has not been demonstrated and the risk of gene transfer from Roundup Ready® canola to other brassicaceous species is therefore considered negligible.

To other organisms

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 and the overall risk is considered negligible.

Herbicide Resistance

The Australian Pesticides and Veterinary Medicines Authority (APVMA) operates the national system that evaluates, registers and regulates agricultural and veterinary chemical products. Both the OGTR and the APVMA recognise the importance of assessing potential risks associated with the use of herbicides on genetically modified canola. In particular, over the

past year, both agencies have been consulting with a range of key stakeholders to evaluate the issues that may arise from the proposed extended use of glyphosate as Roundup Ready® herbicide by Monsanto for weed control in Roundup Ready® canola crops.

The effectiveness of Roundup Ready® canola as a crop depends upon the use of Roundup Ready® herbicide (glyphosate) to control other competing plants and weeds. Because glyphosate has low toxicity to animals (including humans) and microbes, and minimal persistence in the environment, its use is favoured over other, less benign herbicides and may provide an environmental benefit. However, there is potential for development of herbicide-resistant weeds if glyphosate (including Roundup Ready® herbicide) is used (or overused) inappropriately.

Development of herbicide resistance leads to the reduction in options for chemical weed control. In the case of glyphosate resistance, this would mean the reduced usefulness and shortened lifespan of a relatively innocuous, effective and inexpensive agricultural tool. The Regulator is mindful of the importance of glyphosate to Australia in both the agricultural and non-agricultural environments and has worked closely with the APVMA to ensure mechanisms are in place to avoid further development of resistance.

This issue has been assessed by the APVMA and addressed by conditions of registration for the use of Roundup Ready® herbicide on Roundup Ready® canola crops. Accordingly, no specific conditions in relation to management of herbicide resistance are included in the Regulator's licence for Roundup Ready® canola.

The Regulator strongly endorses the range of measures being put in place by the APVMA and industry to minimise the development of herbicide resistance. These measures include:

- implementation of Monsanto's Roundup Ready® canola Resistance Management Plan;
- reporting of resistance incidents to the APVMA; and
- establishment of an industry/expert/government Herbicide Resistance Consultation Group.

Change in Herbicide Use Patterns

During the course of consultations, a number of stakeholders sought clarification on the impact that the introduction of Roundup Ready® canola might have on the herbicides used. It is important to note that mixtures of herbicides are commonly applied to achieve effective control where a range of weeds of differing sensitivity may be present.

Wherever *unwanted* Roundup Ready® canola plants occur (*eg.* following harvest of a Roundup Ready® canola crop or a less likely scenario where glyphosate tolerant weeds develop as a result of gene transfer), methods *other* than glyphosate would have to be used for their eradication. These may include other herbicides or mechanical weed control.

Because glyphosate has low toxicity to animals (including humans) and microbes, and minimal persistence in the environment, its use may provide an environmental benefit over other, less benign herbicides that may be more toxic or persistent (*eg.* able to enter ground water).

The APVMA ensures that the use-pattern associated with these herbicides as specified by label conditions does not compromise the safety of users or the environment and has recently introduced a program for reporting any adverse effects associated with agricultural chemical use. The list of approved chemicals can be reviewed by the APVMA at any time. For example, the herbicide 2,4-D (one of the most commonly used herbicide mixers) and atrazine (the most widely used triazine herbicide) currently under review.

Nevertheless, over-reliance on individual herbicides encourages the development of resistance and there are many other herbicides registered by the APVMA that can be applied. Increasingly, growers are adopting integrated weed management to reduce their reliance on chemicals. This includes measures such as:

- active control of volunteers (both chemical and mechanical);
- informed selection and rotation of herbicides and crops ;
- maintenance of hygiene in seed, harvesting and transport; and
- implementation of good agronomic practice.

In addition to the above measures and those designed to minimise the development of herbicide resistance outlined previously, Monsanto and other industry bodies will be implementing a range of initiatives to promote sustainable agricultural practices generally and integrated weed management practices in particular (see Appendix 4 and 6 for further details). The OGTR and the APVMA are highly supportive of this trend and will continue to liaise to ensure the consistent identification and coordinated management of issues relating to herbicide use and GMOs.

SUMMARY OF THE RISK MANAGEMENT PLAN

Risk of toxicity or allergenicity

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

Risk of weediness

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

Risk of gene transfer

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

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 the GMO or its novel genetic material.

Herbicide resistance

This issue has been assessed by the APVMA and addressed by conditions of registration for the use of Roundup Ready® herbicide on Roundup Ready® canola. Therefore no specific conditions in relation to management of herbicide resistance are included in the Regulator's licence for Roundup Ready® canola. The licence holder's obligation to comply with conditions imposed by the APVMA is noted in the licence.

Reporting conditions

The licence holder is required to provide an annual report on the commercial release. The Act requires all licence holders to inform the Regulator as soon as they become aware of any new information about risks to human health and safety or the environment, or of any unintended effects so that remedial action could be taken. The annual report also includes information on any adverse impacts on human health and safety or the environment caused by the GMO. In addition, Monsanto is required to report to the Regulator the amount of Roundup Ready® canola sold commercially or otherwise grown in each growing season for each State and

Territory. Monsanto is also required to report annually and comply with other conditions required under the APVMA registration of Roundup Ready® herbicide.

Detailed information on the proposed licence conditions is available in the full RARMP document. The RARMP can be obtained from the Office of the Gene Technology Regulator (OGTR) website at www.ogtr.gov.au or by contacting the Office on 1800 181 030.

Industry management proposals

Draft Monsanto guidance documents and industry guidelines developed to assist all participants in the agricultural supply chain to achieve coexistence between different productions systems (*eg.* GM/non-GM) were all considered in the course of the evaluation.

Monsanto's documents aim to achieve effective technology stewardship, and both they and the industry management guidelines focus on agricultural and handling practices which aim to enable separation of GM and conventional crops to the extent required by markets. 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 even in the absence of any supply chain management controls.

Although the evaluation demonstrates there are no risks from Monsanto's Roundup Ready® 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. A number of State and Territory Governments have introduced interim measures pending agreement on market access and supply chain segregation issues. The rate of take-up of Monsanto's Roundup Ready® canola will therefore be determined by State Government and industry consultations.

Although the Regulator has approved the commercial release on human health, safety and environmental grounds, the applicant still needs to obtain the requisite approval from such jurisdictions in order to grow Roundup Ready® canola.

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.

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:	General release of Roundup Ready® canola (*Brassica napus*) in Australia
Applicant:	Monsanto Australia Limited PO Box 6051 St Kilda Rd Central VIC 8008
Common name of the parent organism:	Canola
Scientific name of the parent organism:	*Brassica napus*
Modified traits:	Herbicide tolerance
Identity of the genes responsible for the modified traits:	*CP4 EPSPS* gene from the bacterium *Agrobacterium* sp. (herbicide tolerance) *goxv247* gene from the bacterium *Ochrobactrum anthropi*, formerly *Achromobacter* sp. (herbicide tolerance)
Proposed Location	Potentially all canola growing regions of Australia.
Proposed Size of Release:	Phased introduction (see below) through to full commercial release in all canola-growing regions.
Proposed Date of Release:	From 2003

2. The Office of the Gene Technology Regulator (OGTR) has received an application from Monsanto Australia Ltd (Monsanto) for a licence for the intentional release of a genetically modified organism into the environment. Monsanto proposes the commercial release of GM canola under the trade name Roundup Ready® canola. Monsanto is seeking regulatory approval of one genetically modified 'line'[1] of canola, GT73 (known as RT73 in the USA).
3. Roundup Ready® canola is tolerant to glyphosate, which is the active constituent of a range of proprietary herbicides (registered by the APVMA), including Roundup®. Glyphosate is registered for use in weed control on Roundup Ready® cotton in Australia as 'Roundup Ready® Herbicide by Monsanto' and a parallel application to this one has recently been made for a variation of the registration to enable the extension of use of Roundup Ready® herbicide for use on Roundup Ready® canola (APVMA 2003a).

Section 1.1 The proposed dealings

4. Monsanto proposes the commercial cultivation of Roundup Ready® canola in all the current and potential future canola growing regions of Australia, which includes New

[1] 'Line' is used to describe a GMO with a specific genetic modification derived from a single transformation event and includes the introduction of the genetic modification into other conventional (non-GM) genetic backgrounds by conventional breeding.

South Wales, Victoria, South Australia, Western Australia, Queensland, Tasmania and the Australian Capital Territory. A number of State and Territory governments have introduced or are in the process of introducing, measures to delay the commercial release of certain GM crops until market access and supply chain segregation issues (which by agreement, are outside the scope of the assessment required by the Act) are better understood. Therefore, if the Regulator were to approve the proposed commercial release on human, health, safety and the environment grounds, where necessary the applicant would need to obtain the appropriate approvals, from jurisdictions where it wishes to grow Roundup Ready® canola.

5. Monsanto proposes a phased introduction of Roundup Ready® canola with a limited release of approximately 5000 hectares in the first year in the canola growing regions of south-eastern Australia. Monsanto expects a steady increase in the area sown to Roundup Ready® canola over a number of years across the canola growing regions of Australia, with the rate of increase being determined by market acceptance, seed and variety availability. Monsanto has indicated it intends to continue to work closely with State and Territory Governments and the grains industry, including the Gene Technology Grains Committee, to manage the introduction of Roundup Ready® canola. Monsanto is seeking approval to commence the release as soon as possible.

6. The canola plants and their by-products, would be used in the same manner as conventional canola, including for human food and animal feed. After harvest of the Roundup Ready® canola, the grain will enter the general commerce supply chain in Australia for domestic and export markets. Canola is grown commercially primarily for its seeds that yield about 40% oil and a high protein animal feed. Canola oil is used in the manufacture of a variety of food products. Canola meal is primarily used as a feed for livestock, but it is also used in poultry and fish feed, pet foods and fertilisers.

7. During the processing of (GM and conventional) canola oil, DNA and the vast majority of proteins are removed to a level beyond detection. The use in human food of oil derived from Roundup Ready® canola was approved by Food Standards Australia New Zealand (formerly the Australia New Zealand Food Authority) by inclusion in the Food Standards Code in November 2000 (ANZFA 2000).

8. Monsanto proposes a systematic and strategic approach to risk management and product stewardship through the implementation of its *Roundup Ready® Canola Technology Stewardship Strategy*, which includes a *Roundup Ready® Canola Crop Management Plan*. These will be consistent with the Guidelines for Industry Stewardship Programs and Crop Management Plans proposed by the Plant Industries Committee of the Primary Industries Standing Committee (under the Primary Industries Ministerial Council) and the Guidelines for Supply Chain Management of GM Canola that have been developed by the Gene Technology Grains Committee. Monsanto's documents were draft versions that could not be finalised until regulatory approvals were received from the Regulator and the APVMA. Monsanto has indicated that these documents will be finalised and released in the near future (refer www.monsanto.com.au).

Section 1.2 Parent organism

9. 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/pdf/ir/brassica.pdf).

Section 1.3 Genetic modification and its effects

10. Roundup Ready® canola has been modified to introduce tolerance to the compound glyphosate, the active ingredient in the herbicide Roundup Ready®. Conventional, herbicide tolerant (triazine and imidazolinone) canola varieties currently comprise approximately 60% of the Australian canola market (Norton 2003b).

11. Herbicide tolerance is conferred to Roundup Ready® canola by two mechanisms. The first is through introduction of the *CP4 EPSPS* gene from the soil bacterium *Agrobacterium* sp., which produces a version of an essential plant enzyme that is less sensitive to glyphosate. The second is through the introduction of the *goxv247* gene from the soil bacteria *Ochrobactrum anthropi* that produces glyphosate oxidoreductase, which breaks down glyphosate into non-herbicidal compounds.

12. Short regulatory sequences that control expression of the genes are also present in Roundup Ready® canola. These sequences are derived from the figwort mosaic virus, *Arabidopsis thaliana,* and *Pisum sativum* (Table 1). Although the first organism is a plant pathogen, the regulatory sequences comprise only a small part of their total genome and are not in themselves capable of causing disease.

13. Detailed information on the *CP4 EPSPS* and *goxv247* genes, characterisation of the inserted genetic material and the new proteins expressed by Roundup Ready® canola is provided in Appendix 1.

Table 1: Genetic elements and their origin.

Gene	Promoter	Additional Elements	Terminator
CP4 EPSPS *Agrobacterium* strain CP4	P-CMoVb modified figwort mosaic virus constitutive promoter	AEPSPS/CTP2 *Arabidopsis thaliana* chloroplast transit peptide	E9 3' *Pisum sativum*
goxv247 *Ochrobactrum anthropi* strain LBAA	P-CMoVb modified figwort mosaic virus constitutive promoter	Arab-SSU1A/CTP1 *Arabidopsis thaliana* chloroplast transit peptide	E9 3' *Pisum sativum*

Section 1.4 Method of gene transfer

14. Roundup Ready® canola GT73 was generated by inserting the 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

15. Under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC), Monsanto and Seedex Pty Ltd conducted five limited and controlled releases of Roundup Ready® canola under deliberate release proposals PR-77 (Seedex Pty Ltd, 1997), PR-77X, PR-77X(2), PR-77X(3) and PR-77X(4) (Monsanto, 1998-2001). These trials were carried out in Queensland, New South Wales, Victoria, South Australia, Western Australia, and Tasmania.

16. The first release in Australia of lines covered by this application was in 1997. All previous releases have been carried out under conditions to limit spread or persistence of the GMO in the environment. Roundup Ready® canola GT73 has been grown in various Australian locations and conditions in New South Wales, Victoria, South Australia, Western Australia, Queensland and Tasmania. In the largest approved trial, the planting area was 150 hectares. No adverse effects on human health and safety or the environment were reported for any of these releases.

17. On 22 August 2002 the Regulator issued a licence (DIR 011/2001) to Monsanto for a limited and controlled release of Roundup Ready® canola in two consecutive winter seasons. In the winter of 2002 the total trial area was a maximum of 4 hectares on 4 sites in 3 shires in Victoria and South Australia with no one site having an area greater than 1.2 hectares. In the winter of 2003 the total trial area was 32.8 hectares at a maximum of 25 sites in 13 shires in New South Wales, Victoria, Western Australia and South Australia with no one site having a greater area than 5 hectares.

18. The approvals issued by GMAC and the Regulator included conditions for the management of the trials to minimise the risks posed by the Roundup Ready® canola. Monitoring undertaken by the Interim Office of the Gene Technology Regulator (IOGTR) identified a number of instances of non-compliance with GMAC conditions, as detailed in IOGTR and subsequently OGTR Quarterly Reports; in the trials PR-77X (IOGTR 2001); PR-77X(2) (IOGTR 2000b; IOGTR 2001; OGTR 2002b); PR-77X(3) (IOGTR 2000a; IOGTR 2001); and PR-77X(4) (OGTR 2002c). In some of these instances Monsanto notified the IOGTR of the non-compliance, in others they were detected during IOGTR monitoring activities.

19. Most of the instances of non-compliance related to post-harvest monitoring conditions, in particular the requirement to remove volunteer GM-canola (ie. plants which germinate from fallen seed after the crop has been harvested) from the trial site prior to flowering. In the last of these instances Monsanto elected to destroy the crop when the pollen trap was damaged by herbicide drift, as required by the licence conditions. It should be noted that in each instance the IOGTR or the OGTR (in consultation with GMAC or GTTAC respectively) assessed the risks posed to human health and safety and the environment as a result of the non-compliances as negligible. Monsanto undertook additional management actions to minimise any risks, including removal of volunteers and extension of the monitoring period, for example, as a result of an investigation by the OGTR into instances of non-compliance at sites under PR-77X and PR-77X(2) in Tasmania (see OGTR (2002d, pp 27)

Section 2.2 Approvals by Other Australian Government Agencies

20. 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 Australian Pesticides and Veterinary Medicines Authority (APVMA) and Food Standards Australia New Zealand (FSANZ).

2.2.1 Australian Pesticides and Veterinary Medicines Authority (APVMA)

21. The regulation of herbicides in Australia is the responsibility of the APVMA. Roundup Ready® canola is tolerant to glyphosate, the active constituent of a number of proprietary herbicides including Roundup®. The use of Roundup Ready® canola will enable glyphosate to be used for the control of weeds that emerge following crop planting. Glyphosate is widely used in a variety of formulations for weed control in broadacre agriculture, horticulture and other situations in Australia.

22. Glyphosate is not currently registered for 'in crop' use on canola. A parallel application has been approved by the APVMA for a variation of the registration to enable the extension of use of glyphosate under the trade name 'Roundup Ready® herbicide by Monsanto' for 'in crop' use for weed control on Roundup Ready® canola (APVMA 2003b). Roundup Ready® herbicide is already registered for use on Roundup Ready® cotton in Australia.

23. As there is potential for development of herbicide-resistant weeds if the Roundup Ready® herbicide is used inappropriately, this issue has been assessed by the APVMA and will be addressed by conditions of registration for the herbicide. Feedback to the Regulator from stakeholders also raised the issue of increased use of the herbicide leading to more rapid development of resistance. The Regulator strongly supports the APVMA imposing conditions on the application of herbicide to adequately address both cases of the possible development of glyphosate resistance associated with any extension of use of the Roundup Ready® herbicide to Roundup Ready® canola.

24. 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
APVMA

2.2.2 Food Standards Australia New Zealand (FSANZ)

25. The safety and labelling of foods derived from genetically modified plants are the responsibility of FSANZ. Canola is only consumed by humans as oil in Australia (OGTR 2002a). FSANZ has approved the use of oil derived from Roundup Ready® canola for use in food in Australia by inclusion in the Food Standards code in November 2000 (ANZFA 2000). FSANZ determined that refined oil derived from Roundup Ready® canola is as safe for human consumption as refined oil derived from conventional (non-GM) canola varieties (see Appendix 2). Further details of the risk analysis conducted by FSANZ 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
FSANZ

Section 2.3 International Approvals for Roundup Ready® canola

26. Roundup Ready® canola has been approved for growing and consumption in the US, Canada and Japan and for consumption in Europe.

27. The line GT73 has been approved for food (Table 2), feed (Table 3) and environmental safety (Table 4) are listed below.

Table 2: Food regulatory approvals (oil) obtained for the Roundup Ready® canola

Country	Year Approved
Canada	1994

USA	1995
Japan	1996
European Union	1997
Australia	2000

Table 3: Feed regulatory approvals obtained for obtained for Roundup Ready® canola.

Country	Year Approved
USA	1995
Canada	1995
Japan	1996

Table 4: Environmental regulatory approvals obtained for Roundup Ready® canola.

Country	Year Approved
Canada	1995
Japan	1996
USA	1999
Europe*	Pending

* The European Commission has recently revised the relevant legislation see http://gmoinfo.jrc.it

28. The National Advisory Commission on Agricultural Biotechnology (Comisión Nacional Asesora de Biotecnología Agropecuaria, CONABIA) in Argentina refused an application for a large field trial (500 hectares) of glyphosate-tolerant canola in 1997 (Burachik & Traynor 2002). This decision was based on, Argentina being at the centre of origin of one *Brassica* species and the presence of many other weedy species that are sexually compatible with canola. Additional factors were firstly, concerns regarding the selection of glyphosate-tolerant weeds following the potential increased used of the herbicide, which could only be controlled by less environmentally satisfactory herbicides. Secondly, the apparent intention of the applicant to grow canola off-season to produce bulk seed for export and/or future commercialisation in Argentina (Burachik & Traynor 2002). Therefore agronomic and economic factors which are outside the scope of this assessment contributed to this decision in addition to environmental safety. The issues of weediness and gene flow in the Australian environment are considered in detail in Appendices 4 and 5 respectively. The regulation of herbicide usage is the responsibility of the APVMA but is considered briefly in Appendix 6.

29. Recently (September 2003) the UK Advisory Committee on Releases to the Environment (ACRE, www.acre.org.uk) provided advice to the UK Government recommending against the import of Roundup Ready® canola seed for animal feed, based on the lack of monitoring plans for seed spillage during importation and the apparent anomalous liver weights observed in a rat feeding study (Naylor 1995, Monsanto Unpublished). ACRE indicated that it was 'not fully satisfied at this stage on the basis of the evidence provided that the risk to human health and the environment arising from marketing of this product for importation and processing in the UK will be no different from that of other oilseed rape imported for processing and animal feed purposes' (ACRE 2003b). This represents a reversal of the position of ACRE presented in its primary advice issued 10 March 2003 (ACRE 2003a). Approval to grow Roundup Ready® canola in the UK has not been granted, hence the concern regarding seed spillage. The issue of increased liver weight in rats is considered extensively in Appendix 3, however this risk assessment considers that subsequent studies and the lack of similar findings in other animals refute the original findings by Naylor (1995, Monsanto Unpublished).

30. No other country is known to have refused an application for the release of Roundup Ready® canola on the basis of risks to human health and safety or the environment. There have been no reports of adverse effects on human health or the environment resulting from the use or release of Roundup Ready® canola in Australia or any other countries in which it has been approved.

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

31. 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

32. Comments received from expert groups and key stakeholders consulted on application DIR 020/2002, 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.

33. 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 included:

- the molecular characterisation of the site of insertion of the herbicide tolerance genes in the Roundup Ready® 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 Roundup Ready® canola (Appendix 4 refers);
- the potential for the release to lead to changes in agricultural practices with adverse consequences (Appendices 4 and 6 refer);
- the extent of cross-pollination and gene flow from Roundup Ready ® canola to other canola crops (including other herbicide resistant canola), (Appendix 5 refers);
- the potential for cross-pollination and gene flow to other Brassicas and weedy brassicaceous species with adverse consequences (Appendix 5 refers); and
- whether the new genes introduced into Roundup Ready® canola can transfer to other organisms with adverse consequences (Appendix 5 refers).

34. Issues relating to food safety and herbicide usage, as explained in Chapter 1 Section 2.2, are the responsibility of FSANZ and the APVMA respectively.

35. 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.

36. Submissions received from the consultation on the RARMP, as required by Section 52 of the Act (which included an extended two month period for public comment), also raised a range of issues. 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. A discussion of how issues raised in public consultation on the RARMP were considered is provided in Appendix 10.

SECTION 2 MANAGEMENT OF OTHER ISSUES

37. A number of submissions expressed concern about the possible impact of the commercial release of GM canola on non-GM crops and markets *eg.* 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

38. The GTGC *Canola Industry Stewardship Protocols for Coexistence of Production Systems and Supply Chains* and the applicant's *Roundup Ready® Canola Crop Management Plan* and *Roundup* Ready® *Canola Resistance Management Plan* were both mentioned but not included in the original application from Monsanto. 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.

39. 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.

40. The proposed industry management strategies promote agricultural practice in relation to desired seed purity, cultivation, handling, transport etc. They are designed to preserve the use of Monsanto's technology and enable segregation of GM and conventional (non-GM) canola to the level required by markets, rather than total separation.

41. 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 (ie the risk assessment process considered the risks that might occur in the absence of supply chain management controls).

42. During the assessment Monsanto submitted its draft *Roundup Ready® Canola Crop Management Plan* and associated documents. These were declared 'Confidential Commercial Information' under section 185 of the Act. In accordance with section 184 of the Act this information was not available to the general public. However the information was available to the expert groups that are required to be consulted on the preparation of the RARMP.

43. These documents were draft versions that could not be finalised until regulatory approvals were received from the Regulator and the APVMA. Monsanto has indicated that these documents will be finalised and released in the near future (refer www.monsanto.com.au).

Section 2.2 Role of State and Territory Governments

44. It is important to note that the evaluation of trade, marketing and cost/benefit issues were 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 Roundup Ready® canola to individual farmers, or on market impacts for the agricultural industry.

45. However, these issues are being actively considered by the Australian, State and Territory Governments. The Primary Industries Ministerial Council, which has members from all Australian jurisdictions, has indicted its view that the introduction of GM crops is a matter for industry self-regulation, with oversight by government.

46. All Australian jurisdictions cooperated to develop the gene technology regulatory system and 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 recently issued a policy principle "Gene Technology (Recognition of Designated Areas) Principle 2003" (the Principle), which allows for recognition of GM or non-GM designated areas established under State or Territory legislation for marketing purposes. The Principle is designed to ensure the valid operation of State and Territory laws declaring areas to be GM, non-GM or both for marketing purposes.

47. A number of State and Territory governments have initiated voluntary or legislative measures to ensure the orderly and phased introduction of GM canola into the Australian market. These measures include further examination of the proposed industry segregation procedures and consideration of market effects.

48. Although these arrangements in no way preclude the Regulator approving the commercial release of Roundup Ready® 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

49. In accordance with Section 51 and 52 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 in conjunction with the information provided by the applicant and the body of current scientific information in reaching the conclusions set out in this document.

50. The risk assessment process, detailed in Appendix 9, identified a number of potential hazards that may be posed by the 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, and
- the likely consequences (impact) of the hazard, were it to be realised.

51. 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 4 SUMMARY OF CONCLUSIONS

52. The conclusion of the risk assessment and risk management plan is that the risks to human health and safety and to the environment from the commercial release of Monsanto's Roundup Ready® canola are no greater than those posed by conventional canola. Detailed risk analyses based on the available scientific information are provided in

Appendices 2 - 6 in support of this conclusion. As discussed above, It is important to note that the evaluation of trade, marketing and cost/benefit issues were intentionally excluded from the *Gene Technology Act 2000* assessment process.

53. A range of containment 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, as the risk assessment and risk management plan concludes that the risks to human health and safety or the environment are no greater than conventional canola, no specific containment or supply chain management conditions are included in the licence conditions. However, the Regulator has imposed ongoing reporting conditions that would enable her to proactively review any new information about risks of the proposed release and may amend or add licence conditions accordingly. These are set out in Appendix 8. 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.

54. An issue was identified in relation to the potential for development of herbicide-resistant weeds if the Roundup Ready® herbicide - Roundup Ready® crop combination is used inappropriately. Feedback from stakeholders and the public also raised the issue of inappropriate use of the herbicide leading to resistance. This issue has been assessed by the APVMA and addressed by conditions of registration governing the extension of use of the herbicide on Roundup Ready® canola. Accordingly, no specific conditions in relation to management of herbicide resistance are included in the Gene Technology Regulator's licence for Roundup Ready® canola.

55. The Gene Technology Regulator recognises the importance of glyphosate to Australia in both the agricultural and non-agricultural environments. The Regulator strongly supports APVMA imposing conditions on the registration of glyphosate to address the possibility of resistance development associated with any extension of use of the Roundup Ready® herbicide to Roundup Ready® canola crops.

SECTION 5 DECISION ON THE APPLICATION

56. The conclusion of the risk assessment and risk management plan is that the risks to human health and safety and to the environment from the commercial release of Monsanto's Roundup Ready® canola are no greater than those posed by conventional canola. Accordingly, the Regulator has decided to issue a licence in respect of application number DIR020/2002 with minimal oversight conditions, and which notes the conditions of registration of the herbicide imposed by the APVMA. Detailed risk analyses based on the available scientific information are provided in Appendices 2 - 6 in support of this conclusion.

57. 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.

58. The Regulator must also be satisfied that Monsanto Australia 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 Monsanto Australia 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).

59. Monsanto Australia Ltd is an independently registered company in Australia. After consideration of all the matters under section 58 of the Act and other matters that may affect the applicant's suitability, the Regulator considers Monsanto Australia Ltd is suitable to hold the licence.

Table 1 Summary of the risk assessment and the risk management plan for DIR 020/2002

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
TOXICITY AND ALLERGENICITY FOR HUMANS		See Appendix 2 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 Roundup Ready® canola in human food. The potential for human exposure to the introduced proteins is negligible, as processed canola oil contains negligible amounts of protein or DNA (below the limit of detection).
Toxicity	Negligible	Toxicology studies indicate that Roundup Ready® canola is no more toxic than conventional canola; The novel proteins, CP4 EPSPS and GOX are expressed at low levels in canola leaves and seeds and are both rapidly degraded by mammalian digestive systems; Acute oral toxicity studies demonstrate that the CP4 EPSPS and GOX proteins are not toxic, even at high doses and are not similar to any known toxins; The novel proteins do not share significant sequence homology with known protein toxins; Feeding studies in a range of animals demonstrate that there are no toxic or anti-nutritional effects of the genetic modifications in Roundup Ready® canola; Compositional analyses of Roundup Ready® canola show no significant differences to conventional canola as a result of the genetic modifications; The levels of the naturally occurring toxicants of canola, erucic acid and glucosinolates, are within the range found in conventional (non-GM) varieties and well beneath industry standards; and The introduced GOX protein will metabolise glyphosate to AMPA and glyoxylate. NB The assessment of herbicide residues is the responsibility of the APVMA, and formed part of the consideration of the registration of glyphosate (as 'Roundup Ready® herbicide by Monsanto') for 'in crop' weed control in Roundup Ready® canola. However, the major metabolites of the glyphosate herbicide are not considered toxic, and glyoxylate is a naturally occurring plant metabolite.
Allergenicity	Negligible	Increased allergenicity is unlikely because the novel proteins, CP4 EPSPS and GOX, do not possess a range of characteristics of known allergens. The novel proteins are expressed at low levels in canola leaves and seeds, are rapidly degraded by mammalian digestive systems, and are not glycosylated; The novel proteins are derived from common bacteria that are naturally ubiquitous in the environment and humans are frequently exposed to them; The CP4 EPSPS protein is functionally similar to the endogenous EPSPS occurring in plants. .
TOXICITY AND ALLERGENICITY FOR OTHER ORGANISMS		See Appendix 3 The fact that proteins produced by the introduced genes, CP4 EPSPS and GOX, are naturally occurring in soil microbes, are expressed at low levels, and are not known 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.

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
Vertebrates, including grazing animals, birds and native animals	Negligible	The novel proteins, CP4 EPSPS and GOX are expressed at low levels in plant tissues, do not share significant sequence homology with known protein toxins or allergens, and are rapidly degraded by mammalian digestive systems; The CP4 EPSPS protein is structurally and functionally similar to EPSPS proteins which occur naturally in plants; The levels of the naturally occurring toxicants of canola, erucic acid and glucosinolates, do not vary between GM and conventional canola; Nutritional composition, digestibility and nutritional value of Roundup Ready® canola is not different to conventional canola; Feeding studies in a range of animals (sheep, rats, broiler chickens, quails, trout) demonstrate that there are no toxic or anti-nutritional effects associated with Roundup Ready® canola; Feeding studies with other glyphosate tolerant GM crop plants in a range of animals including pigs, beef and dairy cattle, sheep, poultry and catfish also demonstrate that there are no anti-nutritional effects associated with the presence of the CP4 EPSPS protein in feed; The normal processing of canola seed to produce meal for use in animal feed would be expected to denature CP4 EPSPS and GOX proteins present in Roundup Ready® canola seed; The major metabolites of the glyphosate herbicide are not considered toxic; There are no reports of adverse effects of Roundup Ready® canola on native animals or birds during trials in Australia or commercial production in North America. All these data support the conclusion that the GM canola will not be toxic to agricultural or native animals.
Invertebrates, including insects	Negligible	The introduced proteins CP4 EPSPS and GOX are not considered toxic; Pollen production in Roundup Ready® canola is not different to that in conventional canola; There are no differences between the health of bees foraging on the Roundup Ready® canola or conventional canola; Floral and nectary development is normal in Roundup Ready® canola; There are no reports of adverse effects of Roundup Ready® canola on invertebrates during trials in Australia or commercial production in North America.
Soil biota	Negligible	The introduced genes are derived from commonly occurring soil bacteria and the encoded proteins can be expected to already be present in soil; The proteins produced by the introduced genes are expressed at low levels in Roundup Ready® canola plants; Studies indicate that there are differences between the soil microflora associated with Roundup Ready® canola and other GM and conventional cultivars, but these differences are temporary, not consistently different throughout a season and do not persist between seasons. The observed differences were not associated with any adverse consequences; No adverse impacts on soil microflora have been reported following commercial release in North America or trials in Australia.
WEEDINESS		See Appendix 4
Persistence in the Environment		

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
		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 Roundup Ready® canola will be more likely to persist in the environment and cause more harm to the environment than conventional (non-GM) canola is negligible. There is no evidence to show that the introduced genes increase the potential weediness of the plants. The germination, seed dormancy and fitness traits such as sensitivity to other herbicides, disease resistance, stress adaptation and competitiveness for Roundup Ready® canola fall within the range of conventional canola varieties. The genetic modifications do not provide Roundup Ready® canola with an ecological advantage over conventional canola except in the presence of glyphosate. Glyphosate is widely used for weed control in broad acre agriculture, horticulture and other weed management situations. The APVMA has approved a variation of the registration of glyphosate (as 'Roundup Ready® herbicide by Monsanto') for 'in crop' use on Roundup Ready® canola. 'Roundup Ready® herbicide by Monsanto' was previously registered for use only on Roundup Ready® cotton in Australia. Roundup Ready® canola is only tolerant to glyphosate and its susceptibility to other herbicides is no different to conventional canola. GM volunteers can be managed and controlled using alternative herbicides and other management practices in the same manner as conventional canola volunteers. The impact of such changes is considered to be economic with no adverse impact on human health and safety or the environment.
Agricultural environments	Negligible	See Appendix 4 The risk that Roundup Ready® canola will be more persistent in the agricultural environment than conventional (non-GM) canola and result in a more detrimental environmental impact is negligible. Conventional canola can display secondary dormancy and can persist for several years as an agricultural weed, particularly as volunteers following canola crops resulting from harvest losses; The seed bank of Roundup Ready® canola can be controlled using the same agricultural management practices as for conventional canola; There are no differences between Roundup Ready® canola and conventional canola with respect to the intrinsic characteristics contributing to ecological persistence, such as seed production shattering or dormancy, and competitiveness. Roundup Ready® canola only has a survival advantage in the presence of glyphosate; Glyphosate is commonly used in broadacre cropping for pre-emergent weed control prior to planting. Glyphosate would not be effective in controlling canola volunteers in situations where Roundup Ready® canola had been grown previously; Roundup Ready® canola is as susceptible to all other herbicides except glyphosate as conventional canola and Roundup Ready® canola volunteers can be controlled by using the variety of other herbicides and non-chemical management methods currently used to control conventional canola. Other herbicides may also be incorporated with glyphosate (tank mixing or 'spiking') to ensure control of glyphosate tolerant plants. The presence of Roundup Ready® canola volunteers in agricultural or disturbed habitats will have implications for the choice of herbicide(s) in situations where glyphosate is the principal weed control strategy. As with conventional canola volunteers, Roundup Ready® canola volunteers will represent an agricultural production issue with a potential economic impact in terms of alternative weed management choices, but will pose no greater risks to human health and safety or the environment than conventional canola.

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
Non-cropped disturbed environments	Negligible	See Appendix 4 The risk that Roundup Ready® canola will be more persistent in non-cropped disturbed environments than conventional (non-GM) canola and result in more detrimental environmental impact is negligible. Conventional canola is a minor weed of non-cropped disturbed environments such as roadsides, normally resulting from seed spillage during harvest and transport operations; Conventional canola does not tend to persist in these environments in Australia, and survey observations indicate it does not establish beyond the first few metres adjacent to roads and it is not a good competitor; Canola is not specifically controlled in these situations, though it may be controlled as part of generic weed control operations; Roundup Ready® canola volunteers occurring in disturbed environments will not have any competitive advantage over conventional canola in the absence of glyphosate selection; Glyphosate is widely used in weed control operations in disturbed environments such as roadsides; However while glyphosate is very effective in controlling grasses, it does not always achieve complete control of established broadleaf weeds and a mixture of herbicides (commonly referred to as 'spiking') may be used to ensure complete control of broadleaf weeds; and Roundup Ready® canola volunteers can be controlled using other herbicides or non-chemical techniques currently used for weed control in disturbed environments.
Undisturbed environments	Negligible	See Appendix 4 Conventional canola is not considered a weed of undisturbed environments. It is not considered invasive and it does not persist in undisturbed environments; The risk that Roundup Ready® canola will be more invasive or persistent in undisturbed environments than conventional (non-GM) canola is negligible. Roundup Ready® canola does not have any competitive advantage in the absence of glyphosate and is as susceptible to all other herbicides except as conventional canola; Where herbicides are used to control weeds in undisturbed environments glyphosate is frequently used, but removal is normally by spot spraying, not broadcast spraying, and if Roundup Ready® canola did occur in these environments it could be effectively controlled using other herbicides and non-chemical management techniques.
WEEDINESS – spread in the environment	Negligible	See Appendix 4 The risk that Roundup Ready® canola will be more invasive than conventional (non-GM) canola and spread in the environment, and result in a more detrimental environmental impact is negligible. Conventional canola is primarily dispersed by human activities (harvest, transport) and this would be the case with Roundup Ready® canola; Conventional canola is non-invasive and considered a poor competitor. The genetic modifications do not make Roundup Ready® canola more invasive or persistent in the environment. Roundup Ready® canola does not have any competitive advantage in the absence of glyphosate (usage patterns are discussed in the previous section); Roundup Ready® canola does not differ from conventional canola in growth characteristics in terms of flowering period, pollen production and pollen viability, seed production, seed size, seed germination and dormancy, and agronomic performance, including disease resistance potential and sensitivity to herbicides other than glyphosate; and Seed shattering ability, seed size and seed weight of Roundup Ready® canola were no different to conventional canola lines indicating no alteration in the potential for seed dispersal. Even if Roundup Ready® canola did spread in the environment it can be controlled with herbicides other than glyphosate and non-chemical methods currently used to control canola.

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
GENE TRANSFER – Plants: other canola crops	Negligible	See Appendix 5 Canola is mainly self-pollinated but outcrossing does occur (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; Outcrossing can be detected at greater distances (up to 2.6km under Australian conditions), but at extremely low levels; In a commercial situation low levels of outcrossing between canola varieties is inevitable. If Roundup Ready® canola is grown in close proximity to other canola crops there is a high likelihood of some outcrossing resulting in glyphosate tolerant volunteers in adjacent fields where Roundup Ready® canola has not been grown; Gene transfer from Roundup Ready® canola to conventional seed production plots may result in very low levels of adventitious presence of glyphosate tolerant canola seeds as 'off types' in non-Roundup Ready® canola seed lots. Industry standards for isolation and quality assurance relating to production and marketing of seed for sowing will reduce the likelihood of outcrossing resulting in glyphosate tolerant 'off types' in non-Roundup Ready® canola seed lots. The vast majority of any resultant glyphosate tolerant seeds resulting from outcrossing would be harvested; If gene transfer from Roundup Ready® canola to conventional canola (either commercial or seed crops) did occur as a result of outcrossing, the hazards will be the same as those for Roundup Ready® canola; The possibility of gene transfer from Roundup Ready® canola crops would make the management of canola volunteers more complex and have implications for the choice of herbicide(s) selected for control operations, not only for growers of Roundup Ready® canola, but also for growers of other canola varieties. The 'stacking' of multiple herbicide tolerance traits through outcrossing between Roundup Ready® canola and other herbicide tolerant canola, including GM (glufosinate ammonium tolerant) and conventional (triazine and imidazolinone tolerant) varieties, is also likely to occur at a low frequency, and would also have implications for herbicide choices for the control of canola volunteers; Glyphosate tolerant canola volunteers can be readily controlled by alternative herbicide and non-chemical management practices currently used to control conventional and herbicide tolerant (conventional or GM) canola volunteers; Other herbicides may also be incorporated with glyphosate (tank mixing or 'spiking') to ensure control of glyphosate tolerant plants; The control of glyphosate tolerant canola volunteers that occur as a result of gene flow from Roundup Ready® canola crops represents an agricultural production issue with potential economic impact in terms of alternative weed management choices, but will pose no greater risks of adverse impacts to human health and safety or the environment than conventional canola.
B. napus vegetables and forage rape	Negligible	See Appendix 5 Gene flow is possible from *B. napus* canola to *B. napus* forage rape and vegetables such as swedes, rutabaga and Siberian kale. However, outcrossing would require flowering synchrony and *B. napus* vegetables are generally harvested before flowering; *B. napus* vegetable seed production crops are isolated from other *B. napus* vegetable or canola crops to prevent outcrossing; Forage rape crops rarely flower and are consumed prior to flowering or seed production; If outcrossing and subsequent introgression of the introduced genes from Roundup Ready® canola did occur, the hybrid plants would not have any survival advantage in the absence of glyphosate herbicide; Glyphosate tolerant hybrids can be effectively controlled using a range of alternative herbicides and other non-chemical management techniques currently used for the control of *B. napus* vegetables and forage rape. The novel proteins are not known toxins or allergens.

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
GENE TRANSFER Plants: related *Brassica* species		See Appendix 5 Conventional canola can outcross and form inter-specific hybrids with closely related *Brassica* species *B. rapa*, *B. juncea* and to a lesser extent *B. oleracea*; Introgression (ie. incorporation of genes into a population after an outcrossing event) from canola to *B. rapa* and *B. juncea* can occur.
B. rapa	Very low	*Brassica rapa* is a weed of disturbed and cultivated land and is not found in undisturbed habitats; *B. rapa* is a major weed in Tasmania but its incidence is concentrated in particular geographic locations *B. rapa* is a minor weed of WA, SA, Qld, NSW and Vic; Inter-specific hybrids of canola and *B. rapa* have reduced fertility, seed set and fitness relative to their parents. However recent evidence suggests that hybrids may have increased female fitness and that the reproductive fitness of hybrids is also be influenced by the frequency in resultant populations of both parental species and interspecific hybrids; Low levels of outcrossing and introgression of the introduced genes from Roundup Ready® canola to *B. rapa* populations is likely over time if they are in physical proximity (ie in or adjacent to Roundup Ready® canola crops) and flower in synchrony; 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. If outcrossing and introgression of the introduced genes from Roundup Ready® canola did occur, the inter-specific hybrid plants would not have any survival advantage in the absence of glyphosate herbicide; Glyphosate is a widely used herbicide in weed control in agriculture and other situations, and is one of the options currently used for control of *B. rapa*; Gene transfer to *B. rapa* would not result in an adverse impact on the environment, but it would have implications for the choice of herbicide(s) in situations where glyphosate is the principal strategy for control of this plant; Glyphosate tolerant hybrids can be effectively controlled using a range of alternative herbicides and other non-chemical management techniques currently used for the control of *Brassica* weeds; Glyphosate is not the herbicide of choice for the control of all broadleaf weeds, and other herbicides may also be incorporated with glyphosate (tank mixing and 'spiking') in situations where there is a mixed weed spectrum or enhanced knockdown of difficult to control weeds is required. Tank mixes would provide a management tool for the control of glyphosate tolerant hybrids; Glyphosate would not be used for weed control in or adjacent to paddocks where Roundup Ready® canola has been grown because it would be ineffective in controlling Roundup Ready® canola volunteers. Measures taken to control Roundup Ready® canola volunteers would also eliminate any glyphosate tolerant hybrids; Taking into account the relative weediness, persistence and distribution of this species, the risk of gene transfer to *B. rapa* resulting in adverse impacts on human health and safety or the environment is considered to be very low.

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
B. juncea	Negligible	*Brassica juncea* is an occasional weed of cultivated and disturbed environments and is not found in undisturbed environments; *B. juncea* is an occasional agricultural weed in areas of NSW and Vic; Inter-specific hybrids of canola and *B. juncea* have reduced fertility and seed set; Low levels of outcrossing and introgression of the introduced genes from GM canola to *B. juncea* populations is likely over time if they are in physical proximity and flower in synchrony; If outcrossing and introgression of the introduced genes from Roundup Ready® canola did occur, the inter-specific hybrid plants would not have any survival advantage in the absence of glyphosate herbicide; Gene transfer to *B. juncea* would not result in an adverse impact on the environment, but it would have implications for the choice of herbicide(s) in situations where glyphosate is the principal strategy for control of this plant; Glyphosate tolerant hybrids can be effectively controlled using alternative herbicides and other non-chemical management techniques currently used for the control of Brassica weeds. Glyphosate would not be used for weed control in or adjacent to paddocks where Roundup Ready® canola has been grown because it would be ineffective in controlling Roundup Ready® canola volunteers. Measures taken to control Roundup Ready® canola volunteers would also eliminate any glyphosate tolerant hybrids.
B. oleracea	Negligible	*Brassica oleracea* is not 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.
GENE TRANSFER Plants: other brassicaceous weeds		See Appendix 5 Interspecific hybrids resulting from crosses between conventional canola and the related brassicaceous weed species *Raphanus raphanistrum*, *Hirschfeldia incana* and *Sinapis arvensis* have been observed under natural conditions, but at extremely low frequency. Interspecific hybridisation with other brassicaceous species has not been demonstrated under natural conditions; If outcrossing and introgression of the introduced genes from Roundup Ready® canola did occur, the inter-specific hybrid plants would not have any survival advantage in the absence of glyphosate herbicide; Glyphosate is a widely used herbicide in weed control in agriculture and other situations and is one of the options currently used for control of brassicaceous weeds; Gene transfer to brassicaceous weeds would not result in an adverse impact on the environment, but it would have implications for the choice of herbicide(s) in situations where glyphosate is the principal strategy for control of these weeds; Glyphosate tolerant hybrids can be effectively controlled using a range of alternative herbicides and other non-chemical management techniques currently used for the control of brassicaceous weeds; Glyphosate is not the herbicide of choice for the control of all broadleaf weeds, and other herbicides may also be incorporated with glyphosate (tank mixing or 'spiking') in situations where there is a mixed weed spectrum or enhanced knockdown of difficult to control weeds is required. Tank mixes would provide a management tool for the control of glyphosate tolerant hybrids; Glyphosate would not be used for weed control in or adjacent to paddocks where Roundup Ready® canola has been grown because it would be ineffective in controlling Roundup Ready® canola volunteers. Measures taken to control Roundup Ready® canola volunteers would also eliminate any glyphosate tolerant hybrids. Taking into account the relative weediness, persistence and distribution of these species, the risk of gene transfer to any of these brassicaceous weeds resulting in adverse impacts on human health and safety or the environment is considered to be very low. This is addressed in more detail below.

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
Raphanus raphanistrum	Very low	*R. raphanistrum* occurs in WA, Vic, SA, Qld, NSW and Tas and is a major weed of agriculture in cropping areas of southern Australia; *R. raphanistrum* is also a weed of disturbed areas, but is not considered invasive of undisturbed habitats; 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. Outcrossing would require physical proximity and flowering synchrony, and would be most likely to occur in or adjacent to Roundup Ready® canola crops; 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*
Hirschfeldia incana	Very low	*H. incana* occurs in Qld, NSW, Vic, SA, Tas and WA and is present in disturbed areas of agricultural and native environments; *H. incana* is a minor weed in agricultural areas of Qld and NSW; Inter-specific crossing with canola (conventional or GM) is very unlikely to occur; Outcrossing would require physical proximity and flowering synchrony, and would be most likely to occur in or adjacent to Roundup Ready® canola crops; 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;
Sinapis arvensis	Very low	*S. arvensis* occurs in Qld, Vic, SA, NSW, Tas and WA; *S. arvensis* 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 *S. arvensis* have low vigour and fertility;
GENE TRANSFER - Other organisms		See Appendix 5
Humans	Negligible	Canola oil is the only fraction used as human food; The potential for human exposure to the introduced genes in Roundup Ready® canola is low as processed canola oil contains negligible amounts of DNA or protein (below the limit of detection); Food Standards Australia New Zealand (FSANZ) has approved the use of oil derived from Roundup Ready® canola 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 Roundup Ready® canola to humans is negligible; and Even if gene transfer could occur there would be no adverse consequences, all of the genes are derived from common bacteria that humans are commonly exposed to and do not encode toxins or allergens.
Other Animals	Negligible	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 Roundup Ready® canola to animals is negligible; and Even if gene transfer could occur there would be no adverse consequences, all of the genes are derived from common bacteria and do not encode toxins or allergens.

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
Microorganisms (bacteria, viruses and fungi)	Negligible	Transfer of the introduced genes from Roundup Ready® canola to microorganisms is extremely unlikely; Transfer of DNA from GM plants to soil bacteria has been demonstrated but only under highly artificial laboratory conditions, between homologous sequences, 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 plant viruses has only been demonstrated under controlled conditions between homologous sequences, under conditions of selective pressure and at very low frequency; Transfer of DNA from GM plants to gut bacteria has not been demonstrated under experimental or natural conditions; 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.

Hazard Identification	Risk Assessment (combines likelihood & impact)	Summary Justification of Risk Assessment
Herbicide Resistance	Assessed, addressed by the APVMA	See Appendix 6 Inappropriate use of the Roundup Ready® canola crop – herbicide combination could result in the development of glyphosate resistant weeds through the natural selection of resistant biotypes as a result of the application of the herbicide; Glyphosate is widely used in Australian agriculture, horticulture and for weed control in general. The Regulator recognises that the management of herbicide resistance represents an important issue for agricultural production systems in Australia, especially given the importance of glyphosate to those systems. The Regulator also acknowledges that the introduction of glyphosate tolerant canola, while not representing risks to human health and safety or the environment, has potential implications for herbicide resistance management. Feedback from stakeholders has also raised the issue of inappropriate use of the herbicide leading to resistance. This issue is managed by the APVMA, under conditions of registration for the use of agricultural chemicals in Australia. Conventional canola is sensitive to glyphosate, and this herbicide cannot be used for post-emergent weed control in conventional canola crops (ie once the crop has been planted and germinated). The APVMA has approved a variation of the registration of glyphosate (as 'Roundup Ready® herbicide by Monsanto') for post-emergent weed control in Roundup Ready® canola crops. Monsanto have developed a herbicide resistance management plan for use in conjunction with Roundup Ready® canola; The APVMA's assessment included a consideration of herbicide resistance management and the conditions of registration require the implementation of a herbicide resistance management strategy to address the possibility of resistance development associated with the use of the 'Roundup Ready® herbicide by Monsanto' to Roundup Ready® canola crops; The APVMA has also imposed conditions requiring the reporting of resistant weeds, and auditing and reporting on the implementation of the resistance management strategy. Therefore no specific conditions in relation to management of herbicide resistance are proposed in the Gene Technology Regulator's licence for Roundup Ready® canola. The Regulator strongly supports the conditions imposed by the APVMA on the registration of 'Roundup Ready® herbicide by Monsanto' for Roundup Ready® canola to address the possibility of resistance development. A number of submissions raised the concern that the herbicides likely to be used for the control of Roundup Ready® canola volunteers may be more toxic or more persistent than glyphosate. Such herbicides are registered for use by the APVMA. The APVMA ensures that the use-pattern associated with these herbicides as specified by label conditions does not compromise the safety of users or the environment. The APVMA also have a program to review registered agricultural chemicals that may pose unacceptable risks to people or the environment and a program has recently been initiated for reporting any adverse effects associated with agricultural chemical use. The OGTR and the APVMA will continue to liaise to ensure the consistent identification, evaluation and management of risks associated with the application of agricultural chemicals to GM crops.

APPENDIX 1 INFORMATION ABOUT THE GMO

60. In preparing the risk assessment and risk management plan, the Regulator is required under Section 49 (2) of the Act to consider the properties of the parent organism and the effects of genetic modification.

61. This part of the document addresses these matters and provides detailed information about the GMOs 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.

62. It should be noted that some technical information regarding the precise sequence of the insert has been declared as Confidential Commercial Information (CCI) under Section 184 of the Act. However the information claimed as CCI was made available to the prescribed expert groups that were consulted in the preparation of the risk assessment and risk management plan and this declaration has in no way limited the thorough risk assessment of the genetically modified organism.

SECTION 1 SUMMARY INFORMATION ABOUT THE GMO

Glyphosate (herbicide) tolerance

63. Monsanto Australia Limited (Monsanto) has developed Roundup Ready® canola plants (*Brassica napus*) that are tolerant to glyphosate, which is the active ingredient of a range of proprietary herbicides, including Roundup Ready ® herbicide. Glyphosate is an inhibitor of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS, also known as 3-phosphoshikimate 1-carboxyvinyltransferase), an enzyme of the shikimate pathway for aromatic amino acid biosynthesis that is widespread in plants, fungi, and bacteria.

64. Plants, including weeds, exposed to glyphosate are unable to produce sufficient aromatic amino acids that are essential to their metabolic function, and hence die. The aromatic amino acid biosynthetic pathway is not present in mammalian, avian or aquatic animals. This explains the selective activity in plants and contributes to the low risk to human health and the environment from the use of glyphosate according to label directions.

65. The glyphosate tolerance of Roundup Ready® canola was first demonstrated in field tests conducted throughout the canola growing regions of the United States, Canada, Europe and Australia. Roundup Ready® canola was first planted commercially in 1996 on 50,000 acres in Canada following several field trials starting in 1992. In the 2000 growing-season, approximately 5.4 million acres (2.2 million hectares) of Roundup Ready® canola were planted in Canada and the USA.

The GMO

66. Monsanto is seeking regulatory approval for Roundup Ready® canola line GT73 (also referred as RT73) to be commercially released, in the current and future growing areas of Australia.

67. It should be noted that the descriptor term ‘line’ has been used throughout the risk assessment to denote canola with a specific genetic modification derived from a single transformation event (GT73). This usage is intended to be inclusive of incorporation of the modification into conventional canola genetic backgrounds other than the one that was originally transformed, by conventional breeding.

68. Roundup Ready® canola line GT73 has been approved for food use in Australia by FSANZ (ANZFA 2000).

69. Canola lines containing GT73 are able to tolerate the application of the herbicide glyphosate, the active constituent of a range of proprietary herbicides, including Roundup Ready® herbicide. The proposed dealings involve the commercial release of Roundup Ready® canola lines containing GT73 for use by the Australian canola industry. Roundup Ready® canola material that is harvested would enter general commerce.

70. Roundup Ready® canola line GT73 was genetically modified to contain two genes that each confer higher tolerance to the herbicide glyphosate (N-phosphonomethyl glycine). The *CP4 EPSPS* gene derived from the soil bacterium *Agrobacterium* sp. strain CP4 (Padgette et al. 1995) and the *goxv247* gene derived from the bacterium *Ochrobactrum anthropi* strain LBAA (formerly *Achromobacter* sp. strain LBAA) (Barry et al. 1994, Monsanto Unpublished; Woodward et al. 1994, Monsanto Unpublished).

71. The enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS, also known as 3-phosphoshikimate 1-carboxyvinyltransferase) is a critical enzyme for the production of aromatic amino acids through the shikimate pathway in plants. It catalyses the addition of the enolpyruvyl moiety of phosphoenolpyruvate (PEP) to shikimate-3-phosphate. This enzyme is the target of the herbicide glyphosate by its binding to the enzyme in place of PEP. Inhibition of EPSPS by glyphosate prevents the synthesis of chorismate-derived aromatic amino acids and secondary metabolites (Steinrucken & Amrhein 1980). CP4 EPSPS from *Agrobacterium* sp. strain CP4 is less sensitive to inhibition by glyphosate than the plant enzyme and has been shown to impart tolerance to glyphosate in several crops (Padgette et al. 1993, Monsanto Unpublished).

72. In addition, Roundup Ready® canola produces the enzyme glyphosate oxidoreductase (GOXv247) as a second mechanism to impart tolerance to glyphosate. The GOXv247 protein breaks glyphosate down into the non-toxic compounds aminomethylphosphonic acid (AMPA) and glyoxylate (a common plant metabolite), thus inactivating the herbicide (Pipke & Amrhein 1988). Further details on these two genes and the proteins they encode are provided in Sections 3, 4 and 5.

73. The Roundup Ready® canola plants do not contain any antibiotic resistance genes. The *aad* (Spc^R/Str^R) gene derived from the transposon Tn7, which confers resistance to the aminoglycoside antibiotics streptomycin and spectinomycin (Fling et al. 1985), was present in the plasmid PV-BNGT04 used to transform canola. The *aad* gene was located outside the left and right border sequences on the plasmid and was not transferred into Roundup Ready® canola GT73 by the *Agrobacterium* vector. The *aad* gene was used as a selectable marker gene to enable selection of bacteria containing the plasmid.

74. The Roundup Ready® canola plants to be released are progeny derived from backcrosses of the transgenic canola line GT73 with other canola varieties. The methods used to introduce the genes into canola are discussed in Section 4.

75. An analysis of the potential for Roundup Ready® canola to be a weed and the potential for transfer of genes from the Roundup Ready® canola to other organisms, including weedy brassicaceous relatives, is provided in Appendices 4 and 5 respectively. Appendix 2 discusses the potential for the GMO to be more toxic or allergenic than conventional canola to humans. Appendix 3 analyses the potential for the GMO to be more harmful to other organisms.

SECTION 2 THE PARENT ORGANISM

76. Canola cultivar Westar was transformed in the development of Roundup Ready® canola GT73. Westar was a commercial canola cultivar used in Canada that is now largely superseded.

77. 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 2002). This is available at the OGTR website at www.ogtr.gov.au/pdf/ir/brassica.pdf

SECTION 3 THE INTRODUCED GENES

Section 3.1 The *CP4 EPSPS* gene

78. Roundup Ready® canola GT73 was produced by genetically modifying the parental line by the introduction of the *CP4 EPSPS* gene from the *Agrobacterium* sp. strain CP4, which confers tolerance to glyphosate.

79. The *CP4 EPSPS* gene encodes a protein of 47.6 kD consisting of a single polypeptide of 455 amino acids (Harrison et al. 1993, Monsanto Unpublished). The gene coding for CP4 EPSPS protein is fused with the chloroplast transit peptide-coding region (CTP2) from the *Arabidopsis thaliana epsps* gene (Klee et al. 1987) to target the CP4 EPSPS protein to the chloroplasts (the site of aromatic amino acid biosynthesis). In plants, EPSPS is synthesised as a pre-protein (containing a transit peptide) by free cytoplasmic ribosomes. Transit peptides occur naturally in plants and facilitate the transport of nuclear encoded proteins to the chloroplast. The pre-protein (containing a transit peptide) is transported into the chloroplast stroma where the transit peptide is cleaved and rapidly degraded leaving the mature enzyme (Bartlett et al. 1982; della-Cioppa et al. 1986).

80. Expression of the *CP4 EPSPS* gene is driven by a promoter region from the Figwort mosaic virus, P-CMoVb (Gowda et al. 1989; Richins et al. 1987; Sanger et al. 1990). A promoter is a small piece of DNA that acts like a switch and controls the level of expression of genes. P-CMoVb is a constitutive promoter which directs gene expression in all plant parts (Maiti et al. 1997; Sanger et al. 1990).

81. The P-CMoVb promoter is thought to be equivalent to the 35S promoter from Cauliflower mosaic virus (CaMV), despite low sequence conservation overall between these two promoters. This conclusion was reached because the two promoters occupy similar positions in their respective viral genomes, both increase in strength with increasing sequence length, and the core promoters have significant sequence homology (Sanger et al. 1990).

82. Direct comparison of the Figwort mosaic virus promoter (FLt) with the 35S CaMV promoter has produced conflicting results in different plants for a comparable length of promoter sequence (~300-bp). In tobacco protoplasts FLt was expressed at 2.5 times higher levels than 35S CaMV (Maiti et al. 1997). In *Catharanthus roseus* cells an equivalent FLt promoter produced expression at half the level of the 35S CaMV promoter (van der Fits & Memelink 1997).

83. The FLt promoter showed similar expression in leaves, roots and stems of tobacco, however, evidence suggested expression may be higher in flowers (Sanger et al. 1990). This study (Sanger et al. 1990) used a much longer promoter region (1.1-kb), while expression in flowers from a truncated promoter (~300-bp) was very low (Maiti et al. 1997). This suggests sequences for enhanced flower expression occur in the longer promoter. Separate experiments using a region of the promoter equivalent to the P-CmoVb promoter used in Roundup Ready® Canola GT73 (~600-bp), showed expression from the FLt promoter was equivalent to or higher than 35S CaMV and expression was several fold higher in flowers than in leaves (Rogers 2000).

84. The mRNA polyadenylation signals, which are required for gene expression in plants, are provided by the 3' untranslated region (transcriptional terminator) of the *rbcS E9* gene,

(small subunit 1A ribulose-1,5-biphosphate carboxylase *E9* gene) from pea (*Pisum sativum*) (Coruzzi et al. 1984; Morelli et al. 1985).

85. The DNA sequence of the *CP4 EPSPS* gene from *Agrobacterium* introduced into the Roundup Ready® canola GT73 was altered by site directed mutagenesis to give higher expression in plants. In DNA, nucleotide triplets called codons code for specific amino acids, the basic biochemical units that make up proteins. Some amino acids may be encoded by up to six different codons. The 'bias' of which codon is most frequently used in a gene varies between organisms, with plants often having a different usage to that of bacterial genes. The native *CP4 EPSPS* gene from *Agrobacterium* sp. strain CP4 contains a codon preference that leads to several features that could hinder its expression in canola. These features include sequences encoding potential polyadenylation sites that are rich in A or T nucleotides, a higher G and C nucleotide content than usually found in dicotyledonous plant genes (63% versus ~50%), and concentrated stretches of G and C residues. Alterations were made to the gene sequence by site-directed mutagenesis so that it has a plant preferred codon usage (Padgette et al. 1993, Monsanto Unpublished). Even though the gene sequence has been altered, the protein produced from the plant-preferred gene has the identical amino acid composition to the *Agrobacterium* protein because the changed codon usage still encodes exactly the same amino acids.

Section 3.2 The *goxv247* gene

86. The *goxv247* gene from the bacterium *Ochrobactrum anthropi* strain LBAA encodes the enzyme glyphosate oxidoreductase (GOXv247) which inactivates the herbicide glyphosate by converting it to aminomethylphosphonic acid (AMPA) and glyoxylate (Pipke & Amrhein 1988). Glyoxylate is a common plant metabolite and AMPA is degraded by several microorganisms (ANZFA 2000).

87. The *goxv247* gene encodes a single polypeptide of 431 amino acids with a molecular mass of 46.1 kD. This gene is a variant of the bacterial *gox* gene and has improved affinity for glyphosate and therefore degrades the herbicide more efficiently. The *goxv247* gene varies from the *gox* gene by only 5 nucleotides and the variant GOXv247 protein is 99% identical to the native GOX enzyme, differing by 3 amino acids out of 400 (Woodward et al. 1994, Monsanto Unpublished).

88. The *goxv247* gene was also modified to have a plant-preferred codon usage which was achieved by site-directed mutagenesis (Barry et al. 1994, Monsanto Unpublished).

89. Expression of the gene *goxv247* is driven by the Figwort mosaic virus promoter P-CmoVb (Gowda et al. 1989; Richins et al. 1987; Sanger et al. 1990). The mRNA polyadenylation signals are derived from the 3' untranslated region of *rbcS E9* gene from pea (Coruzzi et al. 1984; Morelli et al. 1985).

90. The gene *goxv247* is fused with a different chloroplast transit peptide (CTP1) sequence derived from the *rbcS* gene of *Arabidopsis thaliana* (Krebbers et al. 1988) to target the GOXv247 protein to the chloroplast (Kolacz et al. 1994, Monsanto Unpublished).

91. The stability and characterisation of introduced genes and expression of CP4 EPSPS and GOXv247 proteins are discussed in Sections 5 and 6.

Section 3.3 Regulatory sequences

92. Although some of the regulatory sequences controlling the introduced genes in Roundup Ready® canola are derived from a plant pathogen, Figwort mosaic virus, these sequences cannot induce disease.

93. The various regulatory sequences controlling the expression of the introduced genes in the Roundup Ready® canola line are summarised in Table 1.

Table 1: Genetic elements in GT73 line and their origin

Gene	Promoter	Additional Elements	3' transcription termination and polyadenylation signals
CP4 EPSPS *Agrobacterium* strain CP4	P-CMoVb modified Figwort mosaic virus constitutive promoter	AEPSPS/CTP2 *Arabidopsis thaliana* chloroplast transit peptide	E9 3' *Pisum sativum*
goxv247 *Ochrobactrum anthropi* strain LBAA	P-CMoVb modified Figwort mosaic virus constitutive promoter	Arab-SSU1A/CTP1 *Arabidopsis thaliana* chloroplast transit peptide	E9 3' *Pisum sativum*

SECTION 4 METHOD OF GENE TRANSFER

94. Roundup Ready® canola GT73 was produced by *Agrobacterium*-mediated transfer (della-Cioppa et al. 1987), using a binary transformation vector PV-BNGT04.

95. The *Agrobacterium*-mediated DNA transformation system is well understood (Zambryski 1992). The plasmid vector, PV-BNGT04 contains well characterised DNA segments required for selection and replication of the plasmid in bacteria as well as *Agrobacterium* sequences essential for DNA transfer from *Agrobacterium* and integration in the plant cell genome (Bevan 1984; Klee & Rogers 1989; Wang et al. 1984).

96. *Agrobacterium 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 *vir* genes on Ti plasmids.

97. Disarmed *Agrobacterium* strains have been constructed specifically for plant transformation. The disarmed strains 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 that produces a crown gall (Klee & Rogers 1989). A useful feature of the Ti plasmid is the flexibility of the *vir* genes to act in either *cis* or *trans* configurations (on the same continuous piece of DNA or on a separate piece) to the T-DNA. This has allowed the development of binary vectors that have the T-DNA and *vir* regions segregated on two plasmids (Bevan 1984).

98. The PV-BNGT04 plasmid used to generate Roundup Ready® canola GT73 contains, between the right and left borders:

- the P-CMoVb promoter from Figwort mosaic virus;
- the chloroplast transit peptide CTP1 sequence of the *rbcS* gene of *Arabidopsis thaliana;*
- the *goxv247* gene derived from the bacterium *Ochrobactrum anthropi;*
- the 3' untranslated region of the *rbcS E9* gene of pea (*Pisum sativum*);
- the P-CMoVb promoter from Figwort mosaic virus;
- the chloroplast transit peptide CTP2 sequence of the *epsps* gene of *Arabidopsis thaliana;*

- the *CP4 EPSPS* gene derived from *Agrobacterium* strain CP4; and
- the 3' untranslated region of the *rbcS E9* gene of pea (*Pisum sativum*).

99. Sequences outside the T-DNA borders of PV-BNGT04 that were NOT transferred included:
    - the ori-V origin of replication from pRK2 for replication in *Agrobacterium tumefaciens;*
    - the ori-322 origin of replication from pBR322 for replication in *Escherichia coli*; and
    - the bacterial *aad* gene derived from transposon Tn7 conferring resistance to the antibiotics streptomycin and spectinomycin enabling propagation and selection of the binary plasmid in *E. coli* and *A. tumefaciens*.

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

100. The genes inserted into genetically modified Roundup Ready® canola GT73 confer a change in phenotype on canola such that the plants gain tolerance to glyphosate. This occurs via two genes that encode two different proteins namely, CP4 EPSPS and GOXv247 (glyphosate oxidoreductase). These two genes are the only genes present in the T-DNA introduced into Roundup Ready® canola GT73.

101. The presence of the *goxv247* and *CP4 EPSPS* genes in Roundup Ready® GT73 canola was confirmed using polymerase chain reaction (PCR) and Southern blot analyses. This demonstrated that only a single copy of the T-DNA was present in the genome of Roundup Ready® canola GT73 at a single location (Kolacz 1994, Monsanto Unpublished). PCR analysis demonstrated that the plasmid backbone sequences, including the antibiotic-resistance marker gene *aad*, are absent in the genome of line GT73. Southern blot analyses performed on inserted DNA, using the *goxv247* gene, *CP4 EPSPS* gene and the E9'3 terminator region as probes from the third (R3) and fifth (R5) generations of Roundup Ready® canola GT73 demonstrated stable inheritance of the inserted DNA. The presence of a single insert was also confirmed by segregation data showing that the glyphosate tolerance phenotype is inherited as a single dominant Mendelian trait (Kolacz 1994, Monsanto Unpublished). Southern data showed single copies of the *CP4 EPSPS* and *goxv247* genes whereas two copies of the E9 3' terminator region are present in Roundup Ready® canola GT73 as expected. The molecular characterisation data of Roundup Ready® canola GT73 is summarised in Table 2.

Table 2: Molecular Characterisation of Roundup Ready® Canola GT73

Line	Gene or Sequence	TRANSGENE INTEGRATION			
		Number of copies	Stable integration and inheritance	Inserted DNA verified by sequencing	Flanking regions determined by DNA sequence
GT73	*CP4 EPSPS*	1	Yes	Yes	Yes
	goxv247	1	Yes	Yes	Yes
	E9 3' terminator	2	Yes	Yes	Yes

102. The DNA sequence of the T-DNA in plasmid PV-BNGT04 was compared with that of the T-DNA insert in Roundup Ready® canola GT73 using the 'Bestfit' algorithm. No differences were detected (Palmer et al. 2003, Monsanto Unpublished).

103. Southern blot analysis was performed to check for the presence of *ori-322* and *ori-V* (origin of replication for bacteria) and for the region containing the bacterial marker gene *aad*, which are present in the backbone of the plasmid PV-BNGT04. No bands were observed when probed with these genetic elements demonstrating the absence of these genetic elements in the Roundup Ready® canola GT73 and further confirming that only the T-DNA contained within the border sequences on the plasmid were integrated.

104. The characterisation of the site of insertion by DNA sequencing also confirms the conclusion that only the T-DNA from PV-BNGT04 was integrated into Roundup Ready® canola GT73. Amplification of DNA by polymerase chain reaction (PCR) using primer pairs located outside the T-DNA borders yielded no PCR fragments. This established that the integration of the plasmid DNA was initiated at the right border and did not proceed outside the left border.

105. The genomic sequences flanking the insert and the sequence of the insertion site in Roundup Ready® canola GT73 and the non-transgenic parental control have been determined (Petersen et al. 2000, Monsanto Unpublished; Rigden et al. 2001, Monsanto Unpublished). These analyses have shown that there is a 22-nucleotide insertion immediately adjacent to the 5' plant-insert junction in event GT73 (Rigden et al. 2001, Monsanto Unpublished). As this 22-bp segment is not present in the corresponding location of the genome in the non-transgenic parental control it is likely that this DNA was introduced at the insertion site during transformation. The 22-bp sequence probably originated from canola DNA. At this same genomic location there is a deletion of 40-bp of plant DNA in event GT73. The deletion and insertion of DNA occurs during *Agrobacterium* transformation due to double-strand break repair mechanisms in the plant (Salomon & Puchta 1998). Rearrangement of DNA at insertion sites has previously been seen in transgenic soybeans (Windels et al. 2002).

106. The company used a computer test for any possible open reading frames (ORFs) across the plant-insert junctions. There was no indication that these proteins would be expressed as none of these ORFs have promoters attached (McCoy and Bannon 2003, Monsanto Unpublished, potential allergenicity of possible peptides discussed in Appendix 2, Section 1.2.2). This extensive characterisation of insertion sites is appropriate for products intended for commercial release.

SECTION 6 EXPRESSION OF THE INTRODUCED PROTEINS

Section 6.1 Identity of the CP4 EPSPS mature protein

107. Molecular analyses (including molecular weight estimation by gel electrophoresis, Western blotting, and N-terminal amino acid sequencing) of the CP4 EPSPS protein in Roundup Ready® canola confirmed that the CTP2 sequence is processed from the CP4 EPSPS protein *in planta* at the predicted cleavage site, yielding a mature protein without any additional amino acids (Harrison et al. 1993, Monsanto Unpublished).

Section 6.2 Identity of the Glyphosate oxidoreductase mature protein

108. The CTP1 sequence directing the GOXv247 protein to the chloroplast contains two possible cleavage sites, 33 and 4 amino acids upstream of the native N-terminus of the GOXv247 protein (Kolacz 1994, Monsanto Unpublished). An attempt to obtain N-terminal amino acid sequence data for GOXv247 from seed of Roundup Ready® canola GT73 was

unsuccessful. However, protein sequence data from GM tobacco plants transformed with a similar *goxv247* gene indicated that CTP1 is cleaved *in planta* such that the mature GOXv247 protein has an additional 4 amino acids at its N-terminus (Kolacz 1994, Monsanto Unpublished). Molecular weight estimation for the GOXv247 protein in Roundup Ready® canola Gt73 by the western blotting technique was consistent with the GOXv247 protein containing 4 additional amino acids. Thus the mature GOXv247 protein expressed in Roundup Ready® canola most likely has 4 additional amino acids at its N-terminus derived from the CTP1 transit peptide (Kolacz 1994, Monsanto Unpublished).

Section 6.3 Expression data from field trials

109. The level of expression of the introduced proteins has been measured in leaf tissue and seeds of Roundup Ready® canola GT73 from three separate field trials, two in Canada and one in Europe. Protein levels were measured by enzyme linked immunosorbent assay (ELISA) (Nickson et al. 1994, Monsanto Unpublished). The results, expressed as µg (microgram) protein/mg fresh weight of plant tissue, are summarised in Table 3. The 1993 and 1994 trials also investigated whether the application of glyphosate altered the level of expression.

110. In the 1992 season (Canada), the seeds and leaves analysed were from plants not treated with herbicide glyphosate. Mean expression levels of CP4 EPSPS and GOXv247 proteins in the leaves were 0.034 and 0.108 µg/mg fresh weight respectively. Mean expression of CP4 EPSPS and GOXv247 in seed were 0.049 and 0.154 µg/mg fresh weight respectively (Nickson et al. 1994, Monsanto Unpublished).

111. Expression levels of novel proteins in the seed during the 1993 (Canada) season were obtained from two separate field studies, one treated with the herbicide Roundup® and another untreated. Mean expression levels for CP4 EPSPS and GOXv247 proteins in untreated and treated Roundup Ready® canola GT73 were 0.028, 0.193 µg/mg fresh weight and 0.030, 0.206 µg/mg fresh weight respectively (Nickson and Taylor 1994, Monsanto Unpublished).

Table 3: Protein Expression Levels in Roundup Ready® canola GT73

Canola line	Plant Material	Protein tested	Mean expression (µg/mg fresh weight)	Range of expression (µg/mg fresh weight)
1992				
GT73 (untreated)	Leaf	CP4 EPSPS GOXv247	0.034 0.108	0.028 - 0.037 0.071 - 0.161
GT73 (untreated)	Seed	CP4 EPSPS GOXv247	0.049 0.154	0.044 – 0.051 0.109 – 0.203
1993				
GT73 (untreated)	Seed	CP4 EPSPS GOXv247	0.028 0.193	0.018 – 0.047 0.108 – 0.334
GT73 (treated, ie. +glyphosate)	Seed	CP4 EPSPS GOXv247	0.030 0.206	0.014 – 0.042 0.125 – 0.379
1994				
GT73 (untreated)	Seed	CP4 EPSPS GOXv247	0.018 0.160	0.016 – 0.022 0.126 – 0.240
GT73 (treated, ie. +glyphosate)	Seed	CP4 EPSPS GOXv247	0.018 0.186	0.012 – 0.022 0.119 – 0.232

1992 (Canadian field trials) – samples taken from 3 of 7 sites;
1993 (Canadian field trials) – means of all samples from 4 sites;
1994 (European field trials) – means of samples from 3 sites.

112. Similarly, protein expression levels in the seed were also obtained from the 1994 (Europe) field studies. Mean expression levels of CP4 EPSPS and GOXv247 proteins in the untreated and treated Roundup Ready® canola GT73 were 0.018, 0.160 µg/mg fresh weight and 0.018, 0.186 µg/mg fresh weight respectively (Taylor 1995, Monsanto Unpublished).

113. No detectable levels of CP4 EPSPS and GOX v247 proteins were obtained in the control Westar canola seed and leaf from all the field trials, as expected.

114. The expression data show that the levels of CP4 EPSPS and GOXv247 proteins in untreated and treated Roundup Ready® canola GT73 plants were similar.

115. These results demonstrate that the introduced proteins CP4 EPSPS and GOXv247 are expressed at very low levels (Table 3). Based on the mean expression levels of all years tested, the level of expression constitutes less than 0.02 % and 0.07% respectively, of the seed on a fresh weight basis.

Section 6.4 Conclusion

116. Monsanto has developed Roundup Ready® canola plants (*Brassica napus*) that are tolerant to glyphosate due to the introduction of the genes *CP4 EPSPS* and *goxv247*. These genes have been well characterised and are stably inherited.

117. The Roundup Ready® canola line GT73 expresses similar levels of CP4 EPSPS and GOXv247 proteins in untreated and treated (glyphosate applied) Roundup Ready® canola GT73 plants. Results from several field trials conducted overseas demonstrate that the introduced proteins CP4 EPSPS and GOXv247 are expressed at very low levels in leaves and seeds. The level of expression constitutes less than 0.02% and 0.07% respectively, of the seed on a fresh weight basis.

APPENDIX 2 HUMAN HEALTH AND SAFETY

118. 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

119. The Roundup Ready® canola GT73 differs from conventional canola in the expression of two additional proteins, CP4 EPSPS and GOXv247. The potential of canola expressing these proteins to be more toxic or allergenic to humans has been considered in detail in this Appendix. These effects would be most likely to occur if the novel gene products were themselves toxins or allergens, if there were unforeseen or unintended effects of the genetic modification or if use of the herbicide on the crop produced toxic or allergenic metabolites.

120. If the genetically modified canola was toxic or allergenic, there could be impacts relating to:

- the safety of human foods containing canola oil (for example cooking and salad oil, margarine, mayonnaise, confectionery products, sandwich spreads, creamers and coffee whiteners);
- the safety of human foods where canola products are present in the food chain (for example livestock, poultry or fish that have been fed canola by-products);
- occupational health and safety (for example, for farm workers, or factory workers involved in canola processing);
- environmental exposure (for example, people breathing canola pollen); and
- toxicity of herbicide metabolites.

121. Responsibility for the assessment of the safety of food for human consumption lies with Food Standards Australia New Zealand (FSANZ, formerly the Australia New Zealand Food Authority, ANZFA). In accordance with the Act, the Regulator seeks advice from FSANZ on all applications in preparing a risk assessment and risk management plan.

122. Oil extracted from Roundup Ready® canola GT73 was approved for use in food for human consumption in Australia and New Zealand by FSANZ in 2000, which concluded that oil from Roundup Ready® canola GT73 is as safe and wholesome as that from other commercially available canola varieties (ANZFA 2000).

123. Responsibility for the assessment of the safety of herbicide metabolites lies with the Australia Pesticides and Veterinary Medicines Authority (APVMA, formerly the National Registration Authority for Agricultural and Veterinary Chemicals, NRA). In accordance with the Act, the Regulator seeks advice from APVMA on all applications for intentional release.

124. The APVMA has approved a variation of the registration of glyphosate (as 'Roundup Ready® herbicide by Monsanto') to enable 'in crop' use on Roundup Ready® canola (APVMA 2003b).

125. Canola has become 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) 1997). Unimproved varieties of *B. napus* (rapeseed)

tend to have high levels of toxic compounds such as erucic acid and alkyl-glucosinolates. Oil suitable for human consumption was first extracted from lines developed in Canada in 1956 (Colton & Potter 1999). Canola is now grown primarily for its seeds, which yield between 35% to over 45% of edible oil. Cooking oil is the primary use but it is present in many other foods. After oil is extracted from the seed the remaining by-product, canola meal, is used as a high protein animal feed.

Section 1.1 Toxicity

126. When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Sjoblad et al. 1992). Acute oral toxicity studies in animals and compositional comparisons to food products known to be safe can provide evidence concerning the toxicity of compounds. Chronic toxicity is not likely to arise from compounds that do not also display the capacity to cause acute toxicity. Apart from the introduced proteins, a toxicity hazard could occur if there was an unintended effect on the plant's metabolism, particularly if this affected erucic acid or glucosinolate levels.

127. In assessing the likelihood of adverse impacts due to toxicity of Roundup Ready® canola, a number of factors were considered, including:

- toxicity of conventional canola;
- the toxicity of the introduced proteins CP4 EPSPS and GOXv247;
- changes to the levels of naturally occurring toxicants, nutritional and anti-nutritional factors; and
- potential for altered metabolism of the herbicide and accumulation of different breakdown products.

128. This appendix presents data and conclusions from:

- acute oral toxicity studies in animals, which provide evidence about the toxicity of the two introduced proteins; and
- compositional studies, which examined fatty acid levels (including erucic acid) in oil, and protein and glucosinolate levels in seed and meal, and compared these to levels in conventional varieties.

129. Roundup Ready® canola GT73 has been assessed by a number of international regulatory agencies with regard to toxicity and allergenicity and subsequently been approved for human use. These include the United States Environmental Protection Agency (EPA 1997; EPA 1996), United States Food and Drug Administration (FDA 1995), United States Department of Agriculture – Animal and Plant Health Inspection Service (USDA-APHIS 1999c; USDA-APHIS 1999d; USDA-APHIS 1998b), Agriculture and Agrifood Canada (Agriculture and Agri-Food Canada (AAFC) 1995), Health Canada (1999), the Organisation for Economic Co-operation and Development (OECD, 1999) and the Japanese Ministry for Health, Labor and Welfare (www.mhlw.go.jp/english/index.html). These agencies have concluded that the presence of EPSPS and GOX proteins in plants released into the environment does not pose a significant toxicity or allergenicity risk and these proteins are now considered inert ingredients (EPA 1997; EPA 1996).

1.1.1 Toxicity of conventional canola

130. Canola seed naturally contains the toxicants erucic acid and alkyl glucosinolates and it is important to determine if the levels of these known toxicants are altered in Roundup Ready® canola GT73. Erucic acid has cardiopathogenic properties and glucosinolates are

considered to be goitrogenic (Verkerk et al. 1998). The term 'canola' refers to those varieties of *B. napus* that meet specific standards defining 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 alkyl glucosinolates of 30 μmoles/g toasted oil free meal) (Organisation for Economic Co-operation and Development (OECD) 2001), and are often referred to as 'double low' varieties (OGTR 2002).

1.1.2 Toxicity of the introduced proteins

131. Canola oil is the only fraction of the commodity used for human food. It is known that refined oils contain negligible amounts of protein (Klurfeld & Kritchevski 1987; Tattrie & Yaguchi 1973). The amount of total protein in the processed fraction of refined, bleached and deodorised (RBD) oil for both Westar and GT73 in the Westar genetic background was below the limit of detection *ie*. less than 0.00013% of oil (Nickson et al. 1994, Monsanto Unpublished).

CP4 EPSPS protein

132. The *CP4 EPSPS* gene is derived from a common soil bacterium, *Agrobacterium* sp. strain CP4 (Padgette et al. 1995), that can be found on plant produce (especially raw vegetables). The CP4 EPSPS protein is functionally similar and structurally identical to EPSPS proteins naturally present in canola and in food and feeds derived from other plant and microbial sources (Stallings et al. 1991)(see also Appendix 1, Section 3.1).

133. The CP4 EPSPS protein is expressed at low levels in seeds and leaves. ELISA analysis of Roundup Ready® canola GT73 and control non-GM Westar seed from trials conducted in 1992, 1993 and 1994 (Nickson et al. 1994, Monsanto Unpublished; Nickson and Taylor 1994, Monsanto Unpublished; Taylor 1995, Monsanto Unpublished), as well as leaf tissue from the 1992 trial (Nickson et al. 1994, Monsanto Unpublished), demonstrated consistently low levels of the introduced protein in these tissues (see Appendix 1 for details).

134. The level of expression of CP4 EPSPS constituted less than 0.02% of the total seed protein on a fresh weight basis in the 1992 trial. Expression of the CP4 EPSPS protein in the seed of Roundup Ready® canola line GT73 was comparable for all trials. The expression of the novel protein in the seed was also comparable for plants treated with the herbicide glyphosate.

135. Western blot analysis of toasted meal showed that the level of CP4 EPSPS was 43.3 - 44.7% of that in the seed prior to processing and this protein did not have any enzymatic activity.

GOXv247 protein

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

137. The GOX protein is expressed at low levels in seeds and leaves. ELISA analysis of Roundup Ready® canola and control non-GM Westar seed from trials conducted in 1992, 1993 and 1994 (Nickson et al. 1994, Monsanto Unpublished; Nickson and Taylor 1994, Monsanto Unpublished; Taylor 1995, Monsanto Unpublished), as well as leaf tissue from the 1992 trial (Nickson et al. 1994, Monsanto Unpublished) demonstrated consistently low levels of the introduced protein in these tissues (see Appendix 1 for details).

138. The level of expression of GOXv247 constituted less than 0.07% of the total seed protein on a fresh weight basis. The expression of the novel protein in the seed was also comparable for plants treated with the herbicide glyphosate. Expression of GOX protein in the seed of Roundup Ready® canola line GT73 was comparable for all trials.

139. The level of GOXv247 protein in toasted meal ranged from and 20.9 - 26% of that in the seed prior to processing and this protein was not found to have any enzymatic activity.

1.1.3 Homology searches

140. The amino acid sequences of both the CP4 EPSPS (Mitsky 1993, Monsanto Unpublished; Harrison et al. 1996) and GOX proteins (Astwood 1995, Monsanto Unpublished) were compared to the amino acid sequences of known protein toxins and no significant homology was found to any known toxin.

1.1.4 Acute toxicity studies

141. Monsanto conducted acute oral toxicity tests using CP4 EPSPS and GOXv247 proteins produced by bacterial expression systems, as the very low expression of these proteins in Roundup Ready® canola GT73 means it is generally not possible to feed test animals a sufficient quantity of the plant material. In this way, it is possible to test the mammalian toxicity of the purified proteins at much higher concentrations than the concentrations produced in the GM plants (FIFRA Scientific Advisory Panel 2000).

142. These proteins were demonstrated to be physically and chemically identical to the proteins produced by the plant by Western blot analysis, N-terminal amino acid sequencing and enzymatic activity (Harrison et al. 1994b, Monsanto Unpublished; Harrison et al. 1994a, Monsanto Unpublished).

143. Mice were gavaged (fed) once with CP4 EPSPS protein at doses of 49 mg/kg, 154 mg/kg and 572 mg/kg and observed twice daily for 7 days after dosing (20 mice per dose, 10 male, 10 female). There were no mortalities and no adverse effects on food consumption, survival, body weight or gross pathology at any of the doses (Harrison et al. 1996). This dose is well above the level of expression of the proteins found in Roundup Ready® canola GT73. Calculated from an average expression level of 0.03 µg/mg CP4 EPSPS in fresh seed (see Appendix 1) the amount of protein the mice (average body weight 30 g) were exposed to at the maximum dose, was equivalent to that in 570g of seed in one dose.

144. Mice gavaged similarly with GOX protein at doses of 1, 10 and 100 mg/kg showed no abnormal effects (Naylor 1994a, Monsanto Unpublished) and the same conclusion was reached using GOXv247 protein at doses of up to 104 mg/kg (Naylor 1994b, Monsanto Unpublished). Again this is a larger dose than that found in the in Roundup Ready® canola GT73 seed. Calculated from an average expression level of 0.18 µg/mg GOXv247 in seed, the amount of protein the mice (average body weight 30 g) were exposed to at the maximum dose was equivalent to that in 17.3 g of fresh seed in one dose.

145. Acute oral toxicity studies in mice support the conclusion that neither the CP4 EPSPS or GOXv247 proteins are toxic, with LD50s of greater than 2500 mg/kg body weight and 5000 mg/kg body weight respectively. Accordingly, longer term, lower dose studies would not be expected to detect any toxic effects.

1.1.5 Feeding studies

146. 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 in rats, lambs, young broiler chickens, bobwhite quails, pigs and trout were undertaken with Roundup Ready® canola GT73 using whole seed and meal. As the only component of canola used in human food is oil and meal is only used in animal food, detailed analysis of these feeding studies are provided in Appendix 3.

1.1.6 Compositional analyses

147. Compositional analysis can provide evidence of whether the genetic modifications have resulted in any unintended effects being introduced into the Roundup Ready® canola GT73; for example, whether there are any significant changes with respect to processing characteristics, oil content, oil composition, oil quality (physical properties), or protein content. Monsanto have provided data from compositional analyses of Roundup Ready® canola GT73 grown in Canada in 1992, 1993, 1998, 2000, Europe (France and the United Kingdom) in 1994 and limited data from Australia.

NUTRITIONAL QUALITIES

148. The nutritional qualities for the Roundup Ready® canola GT73 were determined by compositional analyses of the major components of the seed and meal and were found to be comparable to the non-GM control line Westar. Proximate analyses were done on GT73 canola from field trials in 1992, 1993 and 1994, including analysis on seeds from herbicide treated and untreated plants in 1993 and 1994 (Nickson et al. 1994, Monsanto Unpublished; Nickson and Taylor 1994, Monsanto Unpublished; Nickson et al. 1995, Monsanto Unpublished; Taylor et al. 1996, Monsanto Unpublished). Components measured in seed were protein, fat, moisture, fibre, ash, carbohydrates and calories and the results are presented in Table 1 from the 1992, 1993 and 1994 trials. Data from the 1998 and 2000 Canadian trials are presented in Table 2.

149. In all of the component analyses for all trials of line GT73, there were no significant differences between the glyphosate-tolerant canola and the control line Westar, nor for the seeds from plants treated with herbicide.

*Table 1. Mean values and ranges for the proximate analysis of **canola seed** from three field trials of Westar and GT73.*

Canola Component	Westar[2]		GT73[2]		GT73Treated[4]	
	Mean	Range	Mean	Range	Mean	Range
1992 Canada						
Protein[1]	23.4	21.0-26.1	25.4	25.4-25.7		
Fat[1,2]	46.5	42.3-49.9	45.8	44.6-47.1		
Fibre[1]	8.21	7.16-9.90	7.37	6.26-8.19		
Moisture[3]	4.39	3.69-4.86	4.85	4.32-5.38		
Ash[1]	3.68	3.44-3.91	3.59	3.39-3.79		
Calories Kcal/100g[1]	551	536-567	546	539-554		
Carbohydrate	26.4	23.6-28.0	25.2	23.4-26.9		
1993 Canada						
Protein[1]	23.6	22.8-26.7	23.4	22.3-26.2	23.5	22.7-25.5
Fat[1,2]	45.7	43.3-47.2	46.4	42.7-48.8	46.2	44.3-47.4
Fibre[1]	8.62	8.07-9.59	8.36	7.98-8.77	8.38	8.1-8.94
Moisture[3]	10.4	8.44-11.6	9.22	8.49-9.49	9.67	9.20-10.1
Calories Kcal/100g[1]	513	495-533	523	501-534	520	507-528
Ash[1]	4.07	3.58-4.26	4.00	3.72-4.47	3.93	3.49-4.30
Carbohydrate	26.4	25.8-27.9	26.1	24.9-27.1	26.4	25.7-27.2
1994 Europe						
Protein[1]	27.5	26.3-28.6	25.6	23.9-27.2	25.6	24.5-27.1
Fat[1,2]	39.3	39.0-39.6	42.4	42.1-42.8	43.2	42.3-44.2
Fibre[1]	10.9	10.5-11.2	10.7	10.5-11.0	10.1	9.7-10.6
Moisture[3]	8.30	8.18-8.43	8.43	8.34-8.52	8.63	7.68-9.31
Calories Kcal/100g[1]	495	494-496	510	507-513	512	505-517
Ash[1]	4.83	4.76-4.90	4.26	4.22-4.31	4.25	4.18-4.40
Carbohydrate	28.4	27.6-29.2	27.8	26.4-29.1	24.6	23.0-25.4

[1] Data as a percentage of seed dry weight; [2] 1992 Westar n=7; GT73 n=2; 1993 n=4; 1994 Westar n=2; Untreated GT73 n=2; Treated GT73 n=3; [3] Equilibrium moisture value; [4] 1993 Early post application plot of Roundup® at 0.45 kg active ingredient (a.i.) / ha; 1994 Early post application plot of Roundup® at 2 L/ha.

*Table 2: Compositional analyses of Roundup Ready® **canola seed** and **meal** from 1998 and 2000 Canadian Co-op Trials (Monsanto Company 2002).*

Canola Component	1998				2000			
	Roundup Ready® GT73		Conventional[1]		Roundup Ready® GT73		Conventional[1]	
	Average 15 varieties	Range	Average 15 varieties	Range	Average 36 varieties	Range	Average 36 varieties	Range
Oil (dry basis)	45.3	40.0-51.8	45.4	40.1-51.8	46.7	42.3-55.6	46.3	42.1-53.9
Protein (dry basis)	48.4	41.7-53.7	48.5	41.1-52.7	47.3	38.8-54.0	46.9	40.4-51.1
Saturated fatty acid[2] % total fatty acids	6.9	6.4-7.5	6.8	6.5-7.1	6.7	6.0-8.0	6.6	6.1-7.2
Erucic acid[3] % total fatty acids	0.798	0.00-5.93* (0.00-0.493)	0.397	0.04-0.90	0.08	0.00-0.27	0.05	0.04-0.06
Glucosinolates[4] Micromoles/gram of meal	12.9	7.4-33.3* (7.4-17.9)	14.2	8.6-19.5	9.2	6.7-13.1	10.4	8.1-12.4

[1] Average of 3 non-GM varieties: AC Excel, Defender and Legacy; [2] Saturated fatty acids: (C16:0, C18:0, C20:0, C22:0); [3] Erucic Acid (C22:1) (see below) of planted seed as determined from 2 sub samples of the same sample; [4] Glucosinolates (see below) at 8.5% moisture. * Included lines carrying the Roundup Ready® trait which were derived in part from oilseed rape varieties (OSR) that were not 'double low' canola varieties and which were subsequently discarded (range quoted in brackets is that with these lines excluded).

150. Results of proximate analysis of canola meal samples from the 1992 Canadian field trial are presented in Table 3. No differences were observed between composite seed samples destined for processing into meal, oil and flour across these parameters.

*Table 3. Mean values for the proximate analysis of **toasted meal** composite samples from the 1992 field trial of Westar and Roundup Ready® canola meal (used in rat, trout and bobwhite quail feeding studies, see Appendix 3).*

Canola Component	Westar	Batch 1*	Batch 2†
Protein (% fresh weight)	38.2	39.0	42.2
Ash (% fresh weight)	5.89	6.36	6.55
Moisture (%)	7.16	7.83	5.64
Fat (% fresh weight)	3.79	4.10	3.43
Fibre (% fresh weight)	13.6	12.6	12.3
Carbohydrates (% fresh weight)	45.0	42.7	42.2
Calories (kcal/100g fresh weight)	347	343	347
Nitrogen solubility	19.8	20.0	14.7

* Contained 53% line GT200 and 47% GT73 approx., † Contained 47% line GT200 and 53% GT73 approx.

AMINO ACIDS

151. Amino acid analyses were performed on Roundup Ready® canola GT73 seed from the 1992 Canadian field trial and from untreated and glyphosate-treated plants in 1993. Of the 18 amino acids analysed, the values for each year were comparable for treated or untreated GT73 canola plants and the control line Westar. Some variation between GT73 canola and Westar was observed in these trials however, values were within the reported range for canola (Organisation for Economic Co-operation and Development (OECD) 2001).

152. In the 1993 trials, amino acid values for GT73 were within the ranges determined for Westar. Statistical analysis indicated that in untreated GT73 the mean tryptophan value was significantly different ($p<0.05$) to that for Westar (0.24 g/100g dry seed weight versus 0.26 g/100g dry seed weight). All values however, were within the naturally occurring range for canola, 0.23-0.27g/100g (Organisation for Economic Co-operation and Development (OECD) 2001). The presence of CP4 EPSPS, an enzyme of the shikimate pathway for production of the aromatic amino acids tryptophan, tyrosine and phenylalanine, did not cause an increase in the levels of these aromatic amino acids in Roundup Ready® canola GT73 (Nickson et al. 1994, Monsanto Unpublished; Nickson and Taylor 1994, Monsanto Unpublished). EPSPS is the penultimate enzyme of this multi-step, multi-branched pathway (Herrmann & Weaver 1999). EPSPS enzymes which are not as susceptible to glyphosate, are not as efficient as the plant enzyme in the absence of glyphosate (Schulz et al. 1985) and hence were not be expected to cause an increase in synthesis of aromatic amino acids.

GLUCOSINOLATES

153. Glucosinolates in canola seed are goitre inducing when they are hydrolysed by myrosinase, an enzyme released upon crushing of the canola seed. The processing steps involved in producing canola oil effectively remove glucosinolates from the refined, bleached and deodorised canola oil consumed by humans, however small amounts of glucosinolates remain in the meal. There are over 100 known structural types of glucosinolates, nine of which are monitored in defatted canola meal because of their potentially toxic properties. Five compounds referred to as the alkyl glucosinolates are thought to have anti-nutritional properties. The sum of four of these five alkyl

glucosinolates must be less than a total of 30 μmoles/gram defatted meal for the seed to be classified as canola. Of less concern are the indolyl-glucosinolates, two of which are monitored. Two types from a third group of glucosinolates, the thioalkyl glucosinolates are also measured but are typically present in very low concentrations (ANZFA 2000).

154. Data from analysis of glucosinolates in defatted (toasted) meal from canola line GT73 and the control Westar from the 1992, 1993 and 1994 field trials are shown in Tables 4 and 5. Data from more recent field trials in Canada in 1998 and 2000 are present in Table 2. In 1992 and 1993, analysis was performed by Agriculture Canada using standard methods for the Co-Op Test. The Co-Op Test data (Westar Co-Op) allows comparison of results from GT73 to a much larger data set of values for Westar seed enabling the considerable variation observed in the heterogeneous Westar genotype to be taken into account. In the 1994 trials an alternative technique was used to determine the glucosinolate content, which prevents direct comparison to previous years' values.

*Table 4: Glucosinolate composition of **canola meal** from Westar, Westar Co-Op and glyphosate treated and untreated canola line GT73 grown in Canada in 1992 and 1993.*

Level of glucosinolates (μmoles/g defatted meal)								
Type of glucosinolate	Westar		Westar Co-Op		GT73		Treated GT73[3]	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
1992 Canada[1]								
Alkyl	8.75	6.11-11.4	9.66	7.0-12.5	16.8	13.8-19.8		
Thioalkyl	0.26	0.18-0.40	0.36	0.2-0.8	0.46	0.38-0.55		
Indolyl	11.40	9.8-13.4	11.0	7.0-13.7	11.6	11.55-11.63		
1993 Canada[2]								
Alkyl	8.93	6.7-11.1	7.56	5.3-9.4	10.56	7.97-12.9	10.8	5.57-13.2
Thioalkyl	0.28	0.2-0.37	0.30	0.2-0.4	0.28	0.23-0.33	0.28	0.13-0.37
Indolyl	11.5	11.0-12.5	11.5	10.7-12.5	11.4	10.9-12.0	11.4	10.5-12.5

[1]1992 Westar n=7, GT73 n=2, Co-Op Westar n=13

[2]1993 Westar n=5, Untreated GT73 n=5, Treated GT73 n=5, Co-Op Westar n=9

[3]1993 one application of Roundup® up at 2-4 leaf stage at 0.45 kg a.i./ha

*Table 5: Glucosinolate composition of **canola meal** from Westar and glyphosate-treated and untreated canola line GT73 grown in Europe in 1994.*

1994 Europe	Level of glucosinolates (μmoles/g defatted meal)					
Type of glucosinolate	Westar		GT73		Treated GT73 [1]	
	Mean	Range	Mean	Range	Mean	Range
Alkyl	10.6	9.6-11.4	11.6	9.9-12.9	10.8	9.9-11.6
Indolyl	3.92	3.7-4.3	4.06	3.6-4.4	4.67	4.3-5.1

[1] Treated GT73 had an application of Roundup® at 2L/ha.

155. A comparison of the mean levels of the alkyl glucosinolates in the Roundup Ready® canola GT73 showed that all the values except the 1992 mean value of 16.8 μmol/g were within the range of the Co-Op Test values (7.0-12.5 μmol/g). The level of alkyl glucosinolates in GT73 appeared to be consistently higher than the average determined for Westar, however the higher levels in line GT73 were not attributed to the genetic modification and were consistent with the variability known to occur in the heterozygous canola parent variety. The mean value of 16.8 μmol/g is still well below the maximum limit of 30 μmol/g (Organisation for Economic Co-operation and Development (OECD) 2001).

156. The levels of glucosinolates in Roundup Ready® canola were below the industry standards and did not vary significantly from their parental cultivars or other commercially available canola in the Co-Op test. Independent analysis of GM versus non-GM canola from the 1996 and 1997 seasons in Canada showed glucosinolate levels to be comparable (Daun 1999).

ERUCIC ACID AND OTHER FATTY ACIDS

157. The fatty acid profile is of relevance to the use of canola oil because omega-3 (C18:3, linolenic) and omega-6 (C18:2, linoleic) are essential fatty acids in animal diets. The fatty acid balance also influences the smell and taste qualities of the resulting milk, meat and eggs from livestock. In addition, erucic acid has cardiopathogenic potential and hence canola oil must have less than 2% erucic acid in oil. Oil derived from canola contains only trace amounts of protein and no detectable genetic material.

158. The fatty acid ester profile (which includes an analysis of erucic acid levels) was performed on oil extracted from canola seed harvested from the 1992 and 1993 trials conducted in Canada and the 1994 trial conducted in Europe and is summarised in Table 6. The erucic acid levels from Canadian trials in 1998 and 2000 are shown in Table 2.

159. Except for oleic acid (18:1) levels for each fatty acid are either within the range of Co-Op Westar or the same as that measured in the Westar control. Oleic acid levels in GT73 (treated and untreated) are slightly higher (60.5-64.4) than that observed in Co-Op Westar range (57.4-63.4) and the Westar control (58.8-62.8), however values are still well within industry standards (51.0-70.0, (Organisation for Economic Co-operation and Development (OECD) 2001)). As observed in GT73 oil, erucic acid levels in line GT73 are lower than the Westar line and well below the limit of 2%. In all years, the values for fatty acid esters from GT73 were within the range for Westar from the Co-Op Test except erucic acid, which was below that for Westar. Since canola continues to be bred for lower erucic acid content this is considered to be a positive attribute.

160. An analysis of the RBD oil derived from Roundup Ready® canola GT73 found levels of erucic acid well below the standard for low erucic acid oil at 0.19% of fatty acids (Taylor and Nickson 1995, Monsanto Unpublished). Independent analysis of GM versus non-GM canola from the 1996 and 1997 seasons in Canada showed erucic acid levels to be comparable (Daun 1999).

AUSTRALIAN DATA

161. The composition of canola grown in Australia is not expected to be significantly different from that grown anywhere else in the world. Monsanto provided preliminary data from glucosinolate and erucic acid analysis of a number of Roundup Ready® and non-GM canola varieties grown in Australia. Glucosinolate levels ranged from 5.99-8.44 μmol/g whole seed for Roundup Ready® canola varieties, well within the standard industry limit. Erucic acid was not present at detectable levels in any of the varieties.

Conclusion

162. Analysis of the compositional data of canola seed and toasted meal obtained from the Roundup Ready® canola GT73 indicated that there were no meaningful differences in the levels of major constituents, nutrients, anti-nutritional factors or natural toxicants between GT73 and the control canola line Westar.

*Table 6 Fatty acid ester profiles of extracted **oil** from GT73 and Westar canola from the 1992[1] and 1993[1] and 1994 trials.*

Fatty Acid	% of fatty acid ester profile									
	1992Canada[2]			1993 Canada[3]				1994 Europe[6]		
	Westar	Co-Op	GT73	Westar	Co-Op	GT73	GT73[4] treated	Westar	GT73	GT73[7] treated
16:0 palmitic	3.9-4.2	3.7-4.8	3.98	3.8-4.3	4.0-4.3	4.1	4.1	4.52	4.51	4.50
16:1 palmitoleic	0.3-0.4	0.0-0.6	0.32	0.2[5]	0.2-0.3	0.2	0.2	0.24	0.24	0.24
18:0 stearic	1.4-2.0	1.2-2.1	1.72	1.4-1.9	1.7-1.9	1.7	1.8	1.90	1.5	1.89
18:1 oleic	58.8-62.5	57.4-63.4	61.4	60.1-62.8	61.9-63.1	62.9	62.8	62.6	64.8	64.4
18:2 linoleic	18.9-20.2	18.3-22.1	18.9	18.8-20.6	18.4-19.8	18.7	18.7	20.2	19.0	19.1
18:3 linolenic	8.1-12.1	8.2-13.0	10.8	8.6-10.13	8.5-9.8	9.65	9.73	7.11	6.94	7.00
20:0 arachidic	0.6-0.8	0.4-0.9	0.72	0.6-0.7	0.6-0.7	0.65	0.68	0.77	0.78	0.74
20:1 eicosenoic	1.7-2.0	1.3-2.3	1.58	1.57-2.0	1.4-1.9	1.49	1.51	1.46	1.16	1.17
20:2 eicosadienoic	0.1[5]	0.1-0.2	0.17	0.1[5]	0.1[5]	0.09	0.1	0.1	0.1	0.1
22:0 behenic	0.3-0.4	0.3-0.4	0.40	0.4-0.5	0.4[5]	0.4	0.43	0.36	0.36	0.34
22:1 erucic	0.3-0.6	0.1-1.4	0.12	0.15-0.57	0.04	0.04	0.0	0.32	0.1	0.12
22:2 docosadiencoic								0.1	0.1	0.1
24:0 lignoceric								0.20	0.18	0.18
24:1 nervonic								0.18	0.14	0.15

[1] Analysis by Agriculture and Agri-Food Research Station
[2]Westar n=7; Co-Op Westar n=13; GT73 n=2.
[3]Westar n=15; Co-Op Westar n=8; GT73 n=12; GT73 treated n=15.
[4] Treated with one application of Roundup® at 3-5 leaf stage at 0.45 kg a.i./ha
[5]Single value obtained for all samples.
[6]Westar n=2; Untreated GT73 n=2; Treated GT73 n=3.
[7]Treated with one application of Roundup® at 2 L/ha

1.1.7 Toxicity of herbicide metabolites

163. The potential toxicity of herbicide metabolites is considered by the APVMA in their assessment of a new use pattern for a particular herbicide, in this case glyphosate on Roundup Ready® canola GT73 (APVMA 2003a). This issue will be summarised here. The APVMA has recommended maximum residue limits in appropriate food and animal feed commodities. The residue definition has been amended to include aminomethylphosphonic acid (AMPA), the primary plant metabolite of glyphosate from accelerated degradation by GOX (data supplied by applicant to APVMA).

164. There is no difference in the metabolic fate of glyphosate in Roundup Ready® and conventional canola. However, in glyphosate-sensitive plants very little of the glyphosate that is applied would be broken-down (C. Preston, CRC for Australian Weed Management, pers. comm.). The presence of the GOXv247 protein confers glyphosate tolerance by increasing the rate of breakdown of glyphosate to glyoxylate and AMPA. AMPA is the primary plant metabolite of glyphosate and does not have herbicidal activity (Pipke & Amrhein 1988). Glyoxylate is also a common metabolite in plants and forms part of the biochemical pathway that allows synthesis of carbohydrates from fat (the glyoxylate cycle). AMPA is either non-selectively bound to natural plant constituents, conjugated with naturally occurring organic acids to give trace level secondary metabolites (N-glyceryl-AMPA and N-acetyl-AMPA) or further degraded to one-carbon fragments that are incorporated into a variety of natural products and plant constituents.

165. Glyphosate and AMPA are not teratogenic or developmentally toxic. The acute toxicity of AMPA is low, with an oral LD50 of 8300 mg/kg and the sub-chronic toxicity was also low in studies using rats and dogs (Williams et al. 2000). In rats gavaged with AMPA at a dose of 6.7 mg/kg, the oral absorption of AMPA was low (approximately 20 %). Most AMPA (74%) was eliminated essentially unmetabolised in faeces over a 5-day period. The absorbed AMPA was not metabolised and was excreted rapidly in urine (approximately 65% of the absorbed dose was eliminated in the urine within 12h, and essentially 100% was excreted between 24 and 120 h). Five days after dosing, only trace residues (3 to 6 parts per billion) were detected in the liver, kidney and skeletal muscle (Williams et al. 2000).

166. The APVMA has stated that it is satisfied that any residues resulting from the use of this product, in accordance with label instructions, will not change the food safety of the crop on which it is used (APVMA 2003a).

1.1.8 Other Roundup Ready® crops

167. A number of other crop plants including plants used directly in human food, have been modified to confer glyphosate tolerance by the introduction of the *CP4 EPSPS* including sugar beet, soybeans, corn (maize) and cotton. Additionally, sugar beet, maize and canola have been modified by the addition of both *CP4 EPSPS* and *goxv247*. Safety testing on these crops is discussed in more detail in Appendix 3.

Section 1.2 Allergenicity

168. An allergic response can have severe consequences for an individual and is mediated through the immune system by IgE antibodies, resulting in the release of histamine and other allergic mediators. Predicting allergenicity is difficult and has been based on sequence, structural and biochemical comparisons with known allergens. Protein allergens usually share a number of characteristics (ANZFA 2001; Flavell et al. 1992; Fuchs et al. 1993c; Fuchs et al. 1993b; Fuchs & Astwood 1996; Metcalfe et al. 1996; Taylor 1995; Fuchs et al. 1993a; Davies 1986), including the following:

- 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
- present as the major protein component in the specific food.

Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases (Astwood et al. 1996). This is because it is necessary that a protein is sufficiently stable to reach and cross the mucosal membrane for it to stimulate an allergenic response following oral ingestion (Kimber et al. 1999).

In assessing the likelihood of adverse impacts due to allergenicity of Roundup Ready® canola GT73, a number of factors were considered, including:

- allergenicity of conventional canola;
- allergenicity of the new proteins expressed (CP4 EPSPS and GOXv247); and
- likely levels and routes of exposure to Roundup Ready® 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.

1.2.1 Allergenicity of conventional canola

169. Occupational exposure to canola pollen (Chardin et al. 2001; OGTR 2002), canola dust (Suh et al. 1998) and canola flour (Monsalve et al. 1997; Alvarez et al. 2001) have been implicated in allergic reactions in 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.

1.2.2 Homology with known allergens

170. Amino acid sequence homology has been used as the basis for predicting allergenicity (Gendel 2002; Hileman et al. 2002; Ivanciuc et al. 2002). Searches of various sequence databases have shown no significant matches of CP4 EPSPS and GOXv247 proteins with 1935 known protein toxins present in the Pirprotein, Swissprot and Genpept protein databases (Mitsky 1993, Monsanto Unpublished). The peptide sequences of CP4 EPSPS and GOXv247 proteins were compared with known peptide allergen sequences using the FASTA algorithm and no meaningful homologies were found.

171. A second method for detecting possible allergenic epitopes is based on searching for homology with six to eight amino acids of IgE epitopes known to induce an allergenic response (Kleter & Peijnenburg 2002; Hileman et al. 2002). Using a 6 amino acid window this method was applied to the amino acid sequences of CP4 EPSPS and GOXv247 (Kleter & Peijnenburg 2002). Such searches revealed CP4 EPSPS shared some homology with an allergen from the housedust mite but no homology was found with part of any known IgE epitope. Homology was found between GOXv247 and five IgE epitopes from crustaceans, however it should be noted that a six amino acid match may not be functionally significant because this approach cannot predict whether the putative epitope is on the surface of the protein. There is still scientific debate about the appropriate length of amino acids that should be used in these searches. A recent report suggests that using an epitope of six

amino acids may result in a high rate of random matches and may identify many proteins known not to be allergenic (Hileman et al. 2002).

172. In another study, any putative novel peptides that could be produced across the plant-insert junctions in Roundup Ready® canola GT73 were investigated using the FASTA algorithm and an eight amino acid window (McCoy and Bannon 2003, Monsanto Unpublished). Seven potential ORFs were identified at the 5' end of the insert and 6 potential ORFs at the 3' end of the insert. However, this analysis identified no significant matches between the possible peptides produced from these ORFs and the amino acid sequence of any known toxin or allergen.

1.2.3 Allergenicity of the introduced proteins

The CP4 EPSPS protein

173. The CP4 EPSPS protein is not a known allergen and is not derived from a known source of allergens. Although its molecular weight of ~48 kD is within the range of molecular weights usually shown by allergens, it lacks glycosylation sites (Harrison et al. 1994b, Monsanto Unpublished; Harrison et al. 1994a, Monsanto Unpublished). Glycosylation of a protein involves addition of sugars to the peptide backbone and known allergens are frequently glycosylated.

174. Protein allergens must be stable in the peptic and acidic conditions of the digestive system if they are to reach and pass through the intestinal mucosa to elicit an allergenic response. A study of the digestibility of both CP4 EPSPS and GOXv247 proteins in model digestion systems was done using *in vitro* using simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) as mammalian digestion models (Ream et al. 1993, Monsanto Unpublished; Ream et al. 1994, Monsanto Unpublished; Harrison et al. 1996). The exposure of CP4 EPSPS and GOXv247 proteins to SGF and SIF was conducted over a series of timed incubations at 37˚C. The products of the digestion were analysed using gel electrophoresis, Western blot analysis and enzymatic activity assays.

175. The CP4 EPSPS protein was digested by proteases present in the SGF and SIF systems, suggesting that it would not survive peptic and tryptic digestion or the acidic conditions of the mammalian digestive system (Ream et al. 1993, Monsanto Unpublished). From the simulated digestion experiments and Western blot analyses, the CP4 EPSPS protein had a half–life of less than 15 seconds in the gastric system and 10 minutes in the intestinal system. Similar studies using SGF and SIF preparations have yielded similar results (Chang et al. 2003; Harrison et al. 1996).

176. There is evidence that preheating of the CP4 EPSPS protein results in more rapid degradation in the SGF and SIF systems (Okunuki et al. 2002). Extraction of oil from canola seed involves a heating step (Organisation for Economic Co-operation and Development (OECD) 2001), however protein is only present in canola oil in trace amounts (less than 0.00013 %).

The GOXv247 protein

177. Although the GOXv247 protein (46.1 kD) fits the molecular mass criterion recognised for many allergens of 15–70 kD, it is not glycosylated (Harrison et al. 1994b, Monsanto Unpublished; Harrison et al. 1994a, Monsanto Unpublished). The GOXv247 protein was digested by proteases present in the SGF and SIF systems, suggesting that it would not survive peptic and tryptic digestion or the acidic conditions of the mammalian digestive system (Ream et al. 1994, Monsanto Unpublished). The GOXv247 protein had a half–life of less than 30 seconds in the SIF system.

SECTION 2 EXPOSURE TO THE TOXICITY OR ALLERGENICITY HAZARD

178. The only canola product used for human consumption is oil. Humans are unlikely to be exposed to the proteins through the consumption of canola oil because of the stringency of commercial processing in removing plant proteins to below the limit of detection from the final food product.

Section 2.1 Exposure to pollen via honey

179. The possible exposure of humans to honey containing pollen from the Roundup Ready® canola and any implications for allergenicity was also considered. Honey bees are a major pollinator of canola, and hives may be deployed in breeding, seed increase and general canola production (Manning & Boldand 2000; OGTR 2002; Gibbs & Muirhead 1998). Flowering canola is primarily regarded as a breeding source for commercial apiaries in Australia, in contrast to the situation in North America where it is regarded as a major nectar source.

180. The three factors against this plant being a major source of honey in Australia are cool weather in early spring, weaker populated colonies not being capable of storing any large honey crops and use of pesticides on the crop deterring 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 usually be consumed within a few weeks of the blossom finishing. However, in some years some honey would be extracted, although this honey is not regarded as table quality in Australia (D. Sommerville, NSW Department of Agriculture, pers. comm.).

181. 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).

182. 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* antibiotic (kanamycin) resistance 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 or 0.0000025 ppm. Note that the *nptII* gene is not present in Roundup Ready® canola.

183. No data are available on the expression levels of the CP4 EPSPS and GOXv247 proteins in pollen. However, the introduced proteins are expressed at very low levels in the plant tissues (see Appendix 1 for details). These results indicate that even if the introduced proteins were expressed in Roundup Ready® GT73 canola pollen, the level of exposure that might occur from pollen presence in honey is extremely low. Most importantly however, neither CP4 EPSPS nor GOXv247 are considered to be toxic or allergenic. Similar proteins will be commonly encountered in the environment produced by the soil bacteria from which the genes were derived. Therefore the presence in honey of pollen from the GM canola lines would not represent any allergenicity risk to human health or safety.

Section 2.2 Occupational exposure

184. Agricultural workers will be exposed to canola pollen. As noted in Section 1.2.1, conventional canola pollen *per se* is implicated as a source of allergic reactions. However, the preceding sections have demonstrated that neither of the introduced proteins is likely to be an allergen. No data are available on the expression of the CP4EPSPS and GOXv247 proteins in pollen. From analyses of the Figwort mosaic virus promoter some expression of these genes may be likely in pollen. However, the introduced proteins are expressed at very low levels in the plant tissues (see Appendix 1 for details).

SECTION 3 LIKELIHOOD OF THE TOXICITY OR ALLERGENICITY HAZARD OCCURRING

185. It is considered that the risk of Roundup Ready® canola being more toxic or allergenic to humans than conventional (non-GM) canola is negligible because:

- acute oral toxicity studies demonstrate that the CP4 EPSPS and GOXv247 proteins are not toxic, even at high doses;
- CP4 EPSPS and GOXv247 proteins are both rapidly degraded by mammalian digestive systems;
- the novel proteins do not share significant sequence homology with known protein toxins;
- feeding studies demonstrate that there are no anti-nutritional effects of the genetic modifications in the GM canola (see Appendix 3).
- the composition of Roundup Ready® canola GT73 does not differ significantly from conventional canola;
- the levels of the naturally occurring toxicants of canola such as erucic acid and glucosinolates are not different between GM and conventional canola; and
- the major metabolites of glyphosate are not toxic.
- the novel proteins are expressed at very low levels;
- humans are commonly exposed to the CP4 EPSPS and GOXv247 proteins because they are derived from common bacteria and are naturally ubiquitous in the environment;
- canola oil is the only fraction of the commodity that will be consumed by humans and it does not contain significant levels of protein (below the limit of detection), canola seed or meal is not used in human food;

APPENDIX 3 ENVIRONMENTAL SAFETY- TOXICITY TO OTHER ORGANISMS

186. 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 is considered.

SECTION 1 NATURE OF THE POTENTIAL TOXICITY HAZARD

187. Potentially there could be impacts relating to the toxicity of Roundup Ready® canola GT73 to:

- 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).

188. In assessing the potential for adverse impacts due to toxicity of Roundup Ready® canola GT73 for other organisms, a number of factors were considered which were discussed in detail in Appendix 2, including:

- the novel proteins are expressed at very low levels;
- acute oral toxicity studies demonstrate that the CP4 EPSPS and GOXv247 proteins are not toxic, even at high doses;
- CP4 EPSPS and GOXv247 proteins are both rapidly degraded by mammalian digestive systems;
- the novel proteins do not share significant sequence homology with known protein toxins;
- the CP4 EPSPS and GOXv247 proteins are derived from common bacteria and are naturally ubiquitous in the environment;
- the composition of Roundup Ready® canola GT73 does not differ significantly from conventional canola;
- the levels of the naturally occurring toxicants of canola such as erucic acid and glucosinolates are not significantly different between GM and conventional canola; and
- the major metabolites of glyphosate are not toxic.

Section 1.1 Toxicity hazard of the Roundup Ready® canola GT73 for mammals and wildlife, including birds and fish

189. A range of animals may be exposed to the Roundup Ready® canola GT73, 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 grazing animals (eg kangaroos) or feral animals (eg hares or rabbits) are likely to browse on canola plants. It is possible that livestock may 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 the likelihood of exposure for aquatic species may be considered very low.

190. Both the introduced proteins CP4 EPSPS and GOXv247 are derived from bacteria commonly found in the natural environment such as in soil. Therefore, the proteins expressed in Roundup Ready® canola GT73 are ordinarily present in nature and their presence in canola is not expected to present any new toxicity risks to organisms in these environments. The same or similar proteins are common in bacteria and plants and are a normal part of the diets of animals, humans, insects and microbes (USDA-APHIS 1999a). CP4 EPSPS is structurally and functionally equivalent to the EPSPS enzyme found in canola (Stallings et al. 1991).

1.1.1 Safety of feed for livestock

191. 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 capacity (Brennan et al. 1999). Full fat canola seed may also be used directly as feed for some animals (Roth-Maier 1999; Bertin et al. 1999).

192. 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 aglycone metabolites from glucosinolates such as thiocyanates, isothiocyanates and nitriles (Bell 1984; Canola Council of Canada 2001). Around 85% of conventional canola meal available in Australia is produced via solvent extraction, and the remainder is from cold-pressed seed which may contain levels of glucosinolates that are unacceptable for feeding to animals (Queensland Department of Primary Industries 2002).

193. In toasted meal, data were collected that showed that the amount of CP4 EPSPS and GOXv247 proteins was less than 0.008 % and less than 0.0002 % respectively of seed weight. Hence, processing significantly reduces the amount of introduced protein. Additionally these proteins were not found to have any enzymatic activity (Nickson and Taylor 1994, Monsanto Unpublished; Nickson et al. 1994, Monsanto Unpublished). CP4 EPSPS was found to be degraded more rapidly following heating (Okunuki et al. 2002). CP4 EPSPS and GOXv247 proteins are not known toxins nor do they have the properties of toxins (see Appendix 2 for detailed information).

194. 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). Glucosinolates remain in the canola meal after oil extraction while erucic acid is removed with the oil fraction during processing of canola seed. Industry standards require canola meal to be low in glucosinolates (total glucosinolates of 30 μmoles/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).

195. Compositional analyses presented in Appendix 2 demonstrate that there are no significant changes in Roundup Ready® canola GT73 with respect to erucic acid in seed or glucosinolates in the seed or meal. The levels of erucic acid and glucosinolates in Roundup Ready® canola GT73 are below standard levels and do not vary significantly from their parental cultivars or other commercially available canola.

196. Sinapines are a family of choline esters that naturally occur in canola and can be found in canola meal. Sinapines are known to render an unpleasant odour to chicken eggs if the chickens are fed canola meal and so have some significance in the poultry feed industry. They can also serve as an indicator of perturbations in the shikimate pathway of aromatic amino acid biosynthesis, the target site of glyphosate. The analysis for sinapines in canola meal was performed by Agriculture Canada using published methods. The range of values obtained for sinapine content in GT73 (both treated and untreated) was not significantly different from those obtained for Westar, the conventional parental variety (Nickson et al. 1994, Monsanto Unpublished; Nickson and Taylor 1994, Monsanto Unpublished). Mean values were 12.7 mg/g in defatted meal for both GT73 and Westar in 1992 and 15.8 and 15.5 mg/g defatted meal for GT73 and Westar respectively in 1993. These values fell within the range obtained from the literature of 11.7-18.3 mg/g defatted meal (Nickson et al. 1994, Monsanto Unpublished; Nickson and Taylor 1994, Monsanto Unpublished) and are within the recommended range for sinapines in canola meal (6 – 18 mg/g, Organisation for Economic Co-operation and Development (OECD) 2001).

197. Canola meal is rich in many essential minerals such as phosphorous, calcium, magnesium and zinc but the precise content can be influenced by environmental factors. Phytic acid can adversely affect the uptake of these minerals in animal diets. These constituents were assessed in canola GT73 and the control Westar from trials conducted in 1993 (Nickson et al. 1994, Monsanto Unpublished; Nickson and Taylor 1994, Monsanto Unpublished). The calcium, copper, iron, magnesium, manganese, phosphorus, potassium, zinc and phytic acid content of GT73 was comparable to that found in the control Westar canola (Nickson et al. 1994, Monsanto Unpublished; Nickson and Taylor 1994, Monsanto Unpublished).

198. 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.”

1.1.2 Feeding studies in animals

199. 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 have been undertaken with Roundup Ready® canola GT73. Monsanto submitted data from several feeding studies in order to demonstrate the wholesomeness of toasted canola meal.

RATS

200. Monsanto submitted data from three separate one-month feeding studies conducted on rats (Sprague Dawley strain).

201. The first study with rats was conducted using seed material from the 1992 field trials (see Appendix 2 Table 3). Rats were fed either 0, 5 or 15% processed (toasted and defatted) canola meal or unprocessed (ground) seed from Roundup Ready® canola (a mixture of line GT73 and another glyphosate tolerant line GT200, in two batches) or the parental variety Westar (Roundup Ready® canola GT200 was produced by transformation with the identical plasmid construct as GT73. GT200 was approved for environment releases in Canada but was not commercialised. For the first batch there were no differences in body weight gains for rats of either sex fed a diet containing 5 or 15% level of Roundup Ready® canola meal or seed. For the second batch, male rats fed the 15% dietary level of processed or unprocessed meal from the GM canola mixture exhibited a small but significant reduction in weight gain at the end of the study compared with those fed diets containing the control canola meal/seed (Naylor 1994c, Monsanto Unpublished). As this reduction in weight gain was not consistent between both batches of GM canola meal or between the sexes it is unlikely that it was related to the genetic modification of the canola. Unprocessed canola seed is not usually fed to mono-gastric animals as it contains active myrosinase enzymes that convert glucosinolates into toxic compounds (see Appendix 2, Section 1.1.6, Glucosinolates).

202. A second rat feeding study used processed meal (Naylor 1995, Monsanto Unpublished). No significant differences were observed in body weights or body weight gains between groups of rats fed processed meal from either Roundup Ready® canola GT73 or Westar canola (Naylor 1995, Monsanto Unpublished). However, liver weights relative to final terminal body weight were increased approximately 9% for male rats and 16% for female rats fed 15% Roundup Ready® canola GT73 meal compared to 15% Westar meal. No differences were found in the groups of rats fed a diet containing 5% canola meal. This result was based on one batch of each meal sample, was not replicated on multiple groups of rats and did not include a control diet for comparison. Livers appeared normal at gross necroscopy.

203. The increase in liver weight was attributed to the higher level of alkyl glucosinolates in the Roundup Ready® canola GT73 (Nickson & Hammond 2002). Glucosinolates have been linked to enlargement of the thyroid, adrenal gland, kidney and liver in feeding studies using rapeseed (Verkerk et al. 1998). The higher level of glucosinolates in GT73 in the Westar background relative to the Westar control, was a peculiarity of the Roundup Ready® trait in the Westar genetic background (ANZFA 2000). Genetic background is known to affect glucosinolate level in canola (Downey & Robbelen 1989). Subsequent breeding of the trait into other genetic backgrounds has resulted in glucosinolate levels that are comparable with the parental variety used (Nickson & Hammond 2002).

204. In its assessment of Roundup Ready® canola GT73 FSANZ stated that: ‘Glucosinolate levels in canola can vary enormously and can be influenced by growth and environmental factors as well as the variety grown. The cultivar Westar is made up of a heterogeneous plant population, which exhibits enormous variability. The genetically modified line GT73 was developed from a single plant that was selected from this heterogeneous population and the variation observed in glucosinolate content in the genetically modified plant can be expected for any line developed from a single plant selected from this population. Liver weights can vary and this can be an adaptive change that is indicative of a higher level of metabolic activity. Increased liver weight is commonly observed in toxicity studies, when it

is often considered a physiological adaptation (if dose related), that reaches a steady state with continued dosing and is reversible after cessation of treatment' (ANZFA 2000).

205. A third, comprehensive rat feeding study was conducted in 1996 using processed meal from 3 varieties of European low erucic acid oilseed rape (OSR) and 7 varieties of Canadian canola in two independent batches for each variety. This included two batches of Roundup Ready® variety RU3 (progeny of the GT73 line) and two batches of the parental conventional cultivar Alliance. The GT73 trait was incorporated into Alliance by conventional breeding involving 3 rounds of backcrossing to Alliance and several self-pollinated generations (Naylor 1996, Monsanto Unpublished; summarised in Nickson & Hammond 2002). A commercial basal diet was used for comparison that contained no canola meal. OSR or canola meal was incorporated to the rat's diet at 10% by weight over a one-month study period using groups of ten rats per sex per treatment.

206. Terminal body weights for rats of both sexes fed any diet containing canola or OSR meal were equal to or lower than the average for rats fed the control canola-free diet. This effect of canola as a feed for rats has been noted previously in the literature (Smith & Bray 1992; Vermorel et al. 1987; Vermorel et al. 1988). As a consequence of reduced final body weight, relative kidney and liver weights were increased for both sexes fed any diet containing canola meal as opposed to the basal diet, presumably due to the presence of residual glucosinolates in canola and OSR meal. Residual glucosinolates are broken down by gut flora and release compounds with varying levels of toxicity (Nugon-Baudon et al. 1990). Other studies on conventional canola (Vermorel et al. 1988; Smith & Bray 1992) have shown that there is not always a dose relationship between residual glucosinolate level that can be measured and organ weights, due to biological variation in gut flora and liver metabolism of the rats. In addition this study showed considerable batch to batch variation in average response of the test rats indicating differences caused by processing and biological variation. In conclusion, this study showed that average terminal body weight, liver and kidney weights for the Roundup Ready® variety RU3 were equivalent to that found for Alliance. Furthermore the values for RU3 were within the range found for conventional canola and OSR varieties used in the study and also within the range found previously for conventional canola (Smith & Bray 1992).

207. Recently (September 2003) the UK Advisory Committee on Releases to the Environment (ACRE, www.acre.org.uk) provided advice to the UK Government recommending against the import of Roundup Ready® canola seed for animal feed, based in part on the apparent anomalous liver weights observed in the initial rat feeding studies. ACRE indicated that it was "not fully satisfied at this stage on the basis of the evidence provided that the risk to human health and the environment arising from marketing of this product for importation and processing in the UK will be no different from that of other oilseed rape imported for processing and animal feed purposes" (ACRE 2003b). It should be noted that the September 2003 advice provided by ACRE represented a reversal of advice previously provided (March 2003), in which they indicated that they were satisfied that there was no greater risk to human health or the environment than that from conventional canola (on the proviso that further DNA sequence data and proposed handling procedures were submitted). Despite the data provided in Naylor (1996, Monsanto Unpublished), ACRE were not satisfied as it found that the two studies (Naylor 1995, Monsanto Unpublished; vs Naylor 1996, Monsanto Unpublished) "were not equally comparative".

208. The issue of increased liver weight following feeding with canola has been considered by FSANZ which concluded it to be due to increased metabolic activity in the rats (see above). Previous studies have shown increased liver weights to be common in canola

feeding studies (Smith & Bray 1992; Vermorel et al. 1988). A number of deficiencies in the experimental design of the first and second rat feeding studies were corrected in the third study. The second and third rat feeding studies did not compare Roundup Ready® and non-GM canola meal from the same variety. However, the third rat feeding study used canola meal from a progeny Roundup Ready® cultivar and as such this is more similar to the actual cultivars that will be deployed in the field in Australia than GT73 in the Westar genetic background. This study (Naylor 1996, Monsanto Unpublished) found no difference between the response of rats fed Roundup Ready® canola or conventional canola.

209. Taking into account all of the available data, and the findings of other regulatory agencies, including FSANZ, as well as the confounding effects *per se* of feeding canola to rats, it is concluded that the genetic modification has not resulted in increased toxicity to rats.

210. A number of other studies in other test species have been conducted that do not indicate any adverse effects of feeding Roundup Ready® canola and details of these are provided below.

BOBWHITE QUAIL

211. Feeding studies with 30 northern bobwhite quail chicks (*Colinus virginianus*) in three replicate groups of 10, were fed Roundup Ready® canola meal (in two mixed batches of GT73 and GT200 as for the first rat study above, Naylor 1995, Monsanto Unpublished), the parental variety or a basal diet (Campbell et al. 1993, Monsanto Unpublished). Chicks (10 days old) were fed toasted canola meal at a single dietary level of 20% by weight for 5 days and measured for a further 3 days. There were no mortalities or overt signs of toxicity in either treatment or the control group. In the second study (Campbell and Beavers 1994, Monsanto Unpublished) birds were fed Roundup Ready® canola GT73 meal, the Westar control meal or a basal diet using the same method as the first study. No effects were seen on mortality, body weight, feed consumption or behaviour between birds fed the Roundup Ready® canola GT73 and those fed a conventional diet at the end of the study (Campbell and Beavers 1994, Monsanto Unpublished).

RAINBOW TROUT

212. A trout feeding study (*Oncorhynchus mykiss*) detected no consistent differences in feed efficiency or survival in trout fed Roundup Ready® canola meal or conventional canola meal at 5, 10, 15 or 20% by weight of the total dry diet (Brown et al. 1994, Monsanto Unpublished; Brown et al. 2003b). Processed canola meal was used, derived from seed from the 1992 field trials as for the first rat feeding study and the first bobwhite quail feeding study above (Naylor 1995, Monsanto Unpublished). Trout fed the second batch of Roundup Ready® canola showed a dose dependent decline in weight gain and feed efficiency. This was explained by differences in the processing of this batch of Roundup Ready® canola meal which resulted in a lower nitrogen solubility indicating that this batch was over-toasted causing more proteins to precipitate and certain amino acids to become unavailable (Brown et al. 2003b). Low nitrogen solubility has been shown to render soybean meal less nutritious as an animal feed (Dudley-Cash 2003). A second experiment using GT73 meal processed correctly found it to be equivalent to Westar meal as a fish feed. Fish fed lower levels of canola meal generally did better than those fed 20% canola meal, presumably due to the increasing levels of residual glucosinolates, but no differences between Roundup Ready® canola GT73 and conventional canola meal (Westar) were observed (Brown et al. 2003b).

CHICKENS

213. Rapidly growing broiler chickens (*Gallus domesticus*) were used to compare diets containing Roundup Ready® canola GT73, the parental canola line, and six commercially available reference canola lines (Stanisiewski et al. 2001; Stanisiewski et al. 2001, Monsanto Unpublished; Stanisiewski et al. 2002). Commercial broilers reach a market weight of approximately 2-kg in 42 days and are considered to provide a very sensitive test system for adverse effects of diet. Chickens were fed a diet containing 25% canola meal for 7 days followed by a diet containing 20% canola meal for the remaining 35 days of the study. An approximately 50-fold increase in body weight was observed over this time.

214. An extensive number of performance parameters were measured (starting and final live weights, feed consumption, feed efficiency, adjusted feed efficiency, chill weight, percent chill weight, breast weight, percent breast weight, wing weight, percent wing weight, percent thigh weight, percent drum weight, fat pad weight, fat pad as a percentage of live weight, and moisture, protein, and fat for breast and thigh meat). Means obtained were compared at 5% levels of significance. Values obtained were similar across the broilers fed diets of Roundup Ready® canola GT73, parental control canola, and commercially available reference control lines of canola. No differences were observed when comparisons were expressed on a kg/bird basis and all differences were similar to historical ranges observed for these parameters in previous broiler studies. In conclusion the Roundup Ready® canola GT73 is as wholesome as its corresponding parental line and six commercial reference lines regarding its ability to support the rapid growth of broiler chickens.

LAMBS

215. An independent study comparing the effects of feeding canola meal from commercial varieties of canola, Roundup Ready® canola GT73 and its parent variety in barley based diets for lambs showed that the Roundup Ready® canola GT73 meal did not affect apparent digestibility, growth performance and carcass characteristics (Stanford et al. 2002; Stanford et al. 2003). Carcass yield grade was higher for the diets containing conventional canola although selectable meat yield did not differ among treatments involving Roundup Ready® or parental canola varieties. The source of canola did not affect meat tenderness, as determined by shear force or intramuscular fat content. Meat colour differences were not detected. No aspect of digestibility was influenced by canola source. Canola meal prepared from Roundup Ready® canola GT73 did not alter diet digestibility, feed efficiency, growth performance, carcass characteristics, or meat quality in lambs.

SWINE

216. Pigs were fed a grower ration containing 7.5% canola meal until the average pen weight reached 60.5 ± 4 kg and then fed a finisher ration containing 15% canola meal until the average pen weight was 108.6 ± 7.3 kg. Canola meal was either derived from Roundup Ready® canola, the parental variety or one of two commercial varieties (Aalhus et al. 2003). The average daily weight gain, average daily feed intake and feed conversion efficiency were similar between Roundup Ready® canola meal and the parental variety and differences to the commercial varieties were not statistically significant. In addition carcass characteristics and meat quality evaluations were equivalent.

CONCLUSION

217. Feeding studies in rats, bobwhite quail, trout, chickens, lambs, and pigs using canola meal support the conclusion that the genetic modifications in the Roundup Ready® canola

have not resulted in any additional toxicity or anti-nutritional effects and as such Roundup Ready® canola is comparable with conventional canola.

1.1.3 Feeding studies and composition analysis of other Roundup Ready® GM crops.

218. A number of other crop plants including sugar beet, soybean, corn (maize), cotton and wheat have been genetically modified for glyphosate tolerance via the introduction of least a more herbicide tolerant version of the gene EPSPS from a bacterial or plant source (ie. Roundup Ready ®, RR) as shown in Table 1 below.

Table 1: Composition analysis and feeding studies with other Roundup Ready® (RR) Crops

RR Crop	**Event name and introduced gene(s)**	**Animals tested or type of analysis**	**Conclusion**	**Reference**
Corn, forage & grain	NK603 – 2 copies CP4 EPSPS	Composition	All proximate parameters within published values for non-GM corn.	(Ridley et al. 2002)
Corn, grain	RR – EPSPS	Composition and feeding study in **rats**	Nutrient composition and utilisation by rats equivalent for both the RR and non-GM lines of corn.	(Chrenkova et al. 2002)
Corn, forage quality & grain	GA21 – modified maize EPSPS	Composition and feeding study with broiler **chickens**	Composition analyses showed no biologically significant differences. No differences in growth, feed efficiency, adjusted feed efficiency, or fat-pad weights.	(Sidhu et al. 2000)
Corn, grain	NK603 – 2 copies CP4 EPSPS	Broiler **chickens**	No biologically significant differences. Final live weights and feed conversion efficiency comparable between RR-line and commercial conventional varieties.	(Taylor et al. 2003)
Corn, grain	RR – EPSPS	Broiler **chickens**	RR-line nutritionally equivalent to non-GM parent line. Greater differences found between different conventional cultivars.	(Gaines et al. 2001a)
Corn, grain	RR – EPSPS	Swine (**pigs**)	RR-line equivalent to non-GM parent line for digestible energy coefficients. Greater differences found between conventional cultivars.	(Gaines et al. 2001b)
Corn, meal	RR – CP4 EPSPS	Swine (**pigs**)	Average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency not affect by RR-corn. No difference in carcass midline backfat measurements. No effect on longissimus muscle composition compared to that in pigs feed commercial non-GM corn.	(Fischer et al. 2002)
Corn, silage & grain	GA21 – modified maize EPSPS	Feedlot **cattle** (steers)	No difference in the feeding value of silage or grain from RR-corn and its parental line for beef cattle.	(Petty et al. 2001)
Corn, silage and grain	GA21 – modified maize EPSPS; & NK603 – 2 copies CP4 EPSPS	Feedlot **cattle** (steers)	No significant effect on the nutritive quality of corn for finishing steers. No differences in carcass characteristics.	(Erickson et al. 2003)
Corn, silage & grain	GA21 – modified maize EPSPS	Dairy **cattle**	No differences in dry matter intake, 4% fat-corrected milk (FCM) production, milk composition or ruminal degradability between the RR-line and controls.	(Donkin et al. 2003)
Corn, silage	GA21 – modified maize EPSPS	Dairy **cattle**	Mixed RR-corn ration comprised of 45% corn silage derived from GA21 and a GM-insect resistant corn, compared to a non-GM control line. Total yield, 3.5% FCM and milk composition measurements were not different, indicating no overall product difference. All milk samples were negative for the presence of the introduced DNA (or fragments thereof) and introduced protein (limit of detection = 0.1ng/ml).	(Calsamiglia et al. 2003)

RR Crop	Event name and introduced gene(s)	Animals tested or type of analysis	Conclusion	Reference
Corn, silage & grain	NK603 – 2 copies CP4 EPSPS	Swine (**pigs**)	ADG, ADFI and feed efficiency not effected by RR-corn. Carcass measurements (eg longissimus muscle area) were not different between RR-corn and non-GM corn lines.	(Bressner et al. 2002)
Corn, silage & grain	NK603 – 2 copies CP4 EPSPS	Dairy **cattle**	No effect of the RR-diet on milk composition or 4% FCM production efficiency.	(Grant et al. 2003)
Corn, silage & grain	NK603 – 2 copies CP4 EPSPS	Dairy **cattle**	Milk production and milk composition unchanged.	(Ipharraguerre et al. 2003)
Soybean, seed	GTS40-3-2 & GTS61-67-1 – both contain CP4 EPSPS	Composition	All proximate parameters comparable with non-GM soybeans for plants sprayed with glyphosate.	(Taylor et al. 1999)
Soybean, seed & meal	GTS40-3-2 & GTS61-67-1 – both contain CP4 EPSPS	**Rats**, broiler **chickens**, **catfish**, dairy **cattle**	Growth, feed conversion rates (rats, catfish, chickens), fillet composition (catfish), breast muscle and fat pad weights (chickens), milk production, milk composition, rumen fermentation and nitrogen digestibility (diary cattle) were similar between RR-line and control.	(Hammond et al. 1996)
Soybean, seed & meal	RR – CP4 EPSPS	**Chickens** (laying hens)	Antibodies used to detect GM-protein in eggs if present. Whole eggs and egg white negative for presence of GM-protein. Liver samples and faeces of chickens also negative for GM-protein hence the digestive system of the chicken effectively breaks down protein in feed.	(Ash et al. 2000)
Soybean, meal	GTS 40-3-2 – CP4 EPSPS	Swine (**pigs**), growing-finishing	RR-line and non-GM counterparts are equivalent in composition and nutritive value.	(Cromwell et al. 2002)
Soybean, meal	RR – CP4 EPSPS	Product quality of pork	Loin chops from pigs fed RR or non-GM diets cooked for tasting panel. No difference in juiciness, tenderness, flavour, connective tissue or overall acceptance by tasting panel.	(Armstrong et al. 2001)
Cotton, seed	RR – CP4 EPSPS	Dairy **cattle**	Milk yield, milk composition and body condition were comparable between those feed RR and non-GM cottonseed.	(Castillo et al. 2001b);(Castillo et al. 2001a)
Cotton, seed	RR – EPSPS	Composition and digestibility	Bigger variation found between samples collected in different years than between GM and non-GM varieties. In vitro dry matter digestibility not different and minimal differences in nutrient content.	(Bertrand et al. 2002)
Sugar and Fodder Beet, fodder & pulp	RR – CP4 EPSPS	Composition and feeding study with **sheep**	No significant differences found between RR and non-GM varieties for major nutrients or energy value. Feed value for sheep was the same.	(Hvelplund & Weisbjerg 2001)

219. None of these studies demonstrate any affect of the modification on plant composition or failure of the animals studied to grow and develop normally when feed diets containing Roundup Ready® crop plants.

220. Brake & Evenson (2004) recently published a study which investigated whether there were any inter-generational effects of Roundup Ready® soybeans in mice. Four generations of mice were fed a diet composed of 21.35 % of Roundup Ready® soybeans or a similar percentage of conventional soybeans by weight. This was the highest percentage that allowed a balanced rodent diet. There was no difference in the average body weight of the mice fed Roundup Ready® or conventional soybean containing diets at any developmental time point. In addition average litter size was comparable between Roundup Ready® and conventional diets. This parameter is important because DNA damage can cause embryo death and reabsorption in the uterus of mice, which would lead to fewer offspring but this was not found (Sega & Owens 1983). Furthermore, Brake & Everson (2004) looked at testicular development in mice fed Roundup Ready® soybeans compared to conventional soybeans. The mammalian testis is an organ undergoing rapid cell division and so can indicate if there is DNA damage or inhibition of any processes associated with cell division from ingestion of a toxicant. The cell populations present in the testes of four generations of mice were equivalent in those feed Roundup Ready® or conventional soybeans (Brake & Evenson 2004).

1.1.4 Conclusions on toxicity for mammals and wildlife, including birds and fish

221. The genetic modifications introduced to Roundup Ready ® canola GT73 have not resulted in any significant changes in composition, any increased toxicity or anti-nutritional effects and they will be as safe as conventional canola when consumed by livestock or other animals.

222. Data from feeding studies and acute oral toxicity studies of the introduced proteins, compositional analyses, studies demonstrating the lability of the introduced proteins in simulated mammalian digestive systems, and sequence homology analyses (Appendix 2), support the conclusion that the introduced proteins are not toxic and that there are no anti-nutritional effects of the genetic modifications in Roundup Ready ® canola GT73. Based on these data seed, meal or canola oil derived from line GT73 is considered as safe and nutritionally adequate as parent varieties for use in animal feed.

223. While the assessment of the toxicity of the herbicide metabolites to non-target organisms is the responsibility of the APVMA, the major metabolites of glyphosate are not toxic (refer to Appendix 2 for more details).

224. Canola crops may also be grazed by livestock or other wildlife and canola seed might also be consumed. The introduced proteins are present at low levels in leaves and seeds (see Appendix 1). These proteins are naturally present in the environment and are not considered toxins. There is no evidence to suggest that Roundup Ready® canola GT73 could be harmful to livestock or other grazing animals. There have been no reports of any adverse effects from feeding Roundup Ready® canola to livestock since its commercialisation in Canada in 1996.

Section 1.2 Toxicity hazard of Roundup Ready® canola GT73 for invertebrates (including insects), microbes and soil biota

225. A range of organisms interact with canola within agro-ecosystems including pathogens such as blackleg (*Leptosphaeria maculans*), Scelerotinia stem rot (*Sclerotinia* spp.), Phytophthora root rot caused by the fungus *Phytophtohora megasperma* var. *megasperma,* downey mildew (*Peronospora parasitica*) and alternaria leaf spot (*Alternaria brassicae*),

viral diseases including Beet western yellow virus and Cauliflower mosaic virus, both spread by aphids; insect pests such as the redlegged earth mite (*Halotydeus destructor*), blue oat mite (*Penthaleus major*), cutworms (*Agrotis infusa*), aphids (*Brevicorne brassicae* and *Lipaphis erysimi*), Diamond Back moths or cabbage moths (*Plutella xylostella*), heliothis caterpillars (*Helicoverpa punctigera* and *H. armigera*) and Rutherglen bug (*Nysius vinitor*) (Salisbury et al. 1999).

226. Canadian studies showed that like its parent variety, Roundup Ready® canola GT73 remains susceptible to the fungal disease blackleg. Site managers of Roundup Ready® canola field trials in Australia have made similar qualitative observations in comparison to conventional and triazine tolerant canola varieties (information supplied by applicant). This provides indirect evidence that microbial pathogens of canola and probably other microbial species that interact with canola are unlikely to be affected by the GM plant compared to the conventional variety.

227. Canola is widely used in rotation in Australia as a break-crop, as it is not a host for major soil-borne cereal pathogens such as the take-all fungus (*Gaeummannomyces graminis*). Recent studies on conventional canola have also shown that decaying canola roots release biocidal compounds which are toxic to fungal inoculum in the soil (Kirkegaard et al. 1994; Kirkegaard et al. 1998). This is termed 'biofumigation'.

1.2.1 Soil microbes

228. *Ochrobactrum anthropi* strain LBAA (formerly *Achromobacter* sp.), the source of the *goxv247* gene, and *Agrobacterium* sp. strain CP4, the source of the *CP4 EPSPS* gene, are common soil bacteria. Therefore, both of the introduced proteins are expected to be naturally found in the soil.

229. Several Canadian studies have investigated the interaction of Roundup Ready® canola with soil microbes (Dunfield & Germida 2001; Germida et al. 1998; Germida & Siciliano 1999; Siciliano & Germida 1999; Dunfield 2002). These field experiments in Canada to investigated possible shifts in eubacterial and *Pseudomonas* sp. community structures in the rhizosphere (the soil zone that surrounds and is influenced by the roots of plants) and rhizoplane (the root surface) due to the presence of the glyphosate-tolerant canola variety Quest. These studies used fatty acid methyl ester (FAME) profiles and community level physiological profiling (CLPP) to examine the biodiversity of soil microbial populations. Comparisons were made to other canola varieties, including the isogenic parent line Excel. The results revealed slightly altered microbial communities in the rhizosphere of the Roundup Ready® canola GT73. These differences were not sustained after removal of the plants over winter (Dunfield & Germida 2001; Dunfield & Germida 2003). The authors did not propose any hypothesis to correlate the observed microflora differences with the GM-canola. Moreover, these studies did not indicate whether glyphosate was applied during the growth of the Roundup Ready® canola (ACRE 2002).

230. Application of herbicides can affect proportions of soil microbes eg. (Gyamfi et al. 2002; Becker et al. 2001). 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. hygroscopicus* and *S. viridichromogenes* and which also possess the PAT enzyme for detoxification (Hoerlein 1994). Metazachlor is also metabolised by soil microflora (Beulke & Malkomes 2001). Similar effects have been found in glyphosate treated soil (Haney et al. 2002; Araujo et al. 2003).

231. Experiments were conducted in Canada after the 1992 and 1993 field trials of Roundup Ready® canola using a subsequent crop of barley. Plots that had been used the previous year for Roundup Ready® canola cultivation (and sprayed with Roundup®) showed no change in plant count or yield for barley compared with the plots used for the control (data supplied by the applicant). This provides indirect evidence that if there are any allelopathic effects on soil microflora then such effects are transient. This is borne out in recent studies on microbial communities in Roundup Ready® canola in Canada (Dunfield & Germida 2003)

232. It is well known that physical (eg. soil type, climactic conditions, cultivation practices), temporal (seasonal changes, past history of the site) and biological factors (eg. crop type and variety) will affect soil microbial populations. Differences both between crops and between cultivars of the same crop species have been observed (Germida et al. 1998; Siciliano et al. 1998; Bruinsma et al. 2003). The extent of natural variations in the numbers and diversity of soil microbe populations due to these factors remain to be established.

233. Termorshuizen & Lotz (2002) hypothesised that the post-emergent application of herbicide, that is enabled by herbicide-tolerant crops, may increase the levels of opportunistic root pathogens multiplying in the roots of plants killed by the herbicide. Numerous studies have shown increased populations of various fungi in soil sprayed with glyphosate (see Sanogo et al. 2000 and references therein). The observation that conventional bean plants sprayed with glyphosate died more quickly if inoculated with pathogenic fungi was first noted by Johal & Rahe (1984). This phenomenon has also been observed in conventional soybeans sprayed with other herbicides such as imazethapyr, tradename Spinnaker® (Sanogo et al. 2000).

234. Several studies have investigated the relationship between glyphosate and specific diseases in Roundup Ready® soybean cultivars in the US. In glasshouse experiments, glyphosate application increased the level of *Fusarium solani* f. sp. *glycines,* the causal agent of sudden death syndrome of soybeans, on the roots of the soybean plants. However, this study concluded that both Roundup Ready® and conventional soybeans responded similarly to the presence of the fungus on the roots (Sanogo et al. 2000). The level of disease resistance inherent in the cultivar in which the Roundup Ready® trait was incorporated was the determining factor in the severity of the disease symptoms. Other contributing factors were the level of inoculum in the soil and the virulence of the fungal strain (Sanogo et al. 2000). Subsequent field experiments showed no correlation between root colonisation by *F. solani* f. sp. *glycines* and disease symptoms (Njiti et al. 2003). Likewise these authors concluded that the genotype of the cultivar was most important in determining the severity of the disease. Furthermore similar results were found in experiments with *Sclerotinia sclerotiorum*, causal agent of Sclerotinia stem rot and *Rhizotonia solani*, a damping-off pathogen (Nelson et al. 2002; Harikrishnan & Yang 2002).

235. These experiments have indicated that the prevalence of specific fungal diseases is not higher in Roundup Ready® soybean cultivars with genetic resistance to the specific fungal disease. Recent coverage in the popular press has highlighted a report of increased levels of *Fusarium* species in Canadian fields sprayed with glyphosate. This particular claim remains unsubstantiated in the scientific literature. However, as indicated above, this side effect of glyphosate has been well documented and has not inhibited its widespread adoption and use to date of glyphosate based herbicides.

236. Plant residue from Roundup Ready® canola GT73 did not have an impact on the agronomic performance of a succeeding crop of barley and peas (Agriculture and Agri-

Food Canada (AAFC) 1995). There was no significant difference in plant numbers or grain yield between plots where Roundup Ready® canola GT73 and non-GM Westar canola had been grown in the previous year. Agriculture and Agri-Food Canada concluded that this provided indirect evidence that soil microorganisms involved in maintaining soil fertility were not negatively affected by GT73 plant residues in comparison to Westar residues. Past trial sites that contained both GM and non-GM material would allow obvious differences in their capacity to add and remove substances to be detected. A recent study (Prately and Stanton, 2000 unpublished) found that growing of Roundup Ready® canola GT73 does not impact on the weed control or yield of wheat grown on the same plots in the following year compared to conventional or triazine tolerant canola. These studies suggest that the ability of the GM canola line to add or subtract substances from the soil is unchanged. The applicant has stated that there is no evidence to indicate that the biodegradability of the GM canola will be different to that of the parent line Westar and no change in the impact on the soil is expected.

237. Knowledge of the mode of action of the CP4 EPSPS and GOXv247 enzymes, and the lack of known toxicity for the newly expressed proteins, suggests that it is highly unlikely there will be deleterious effects on other organisms (USDA-APHIS 1999a). Roundup Ready® canola GT73 has been grown on a limited scale in Australia since field trials were approved by GMAC in 1997. There have been no reports of adverse effects on beneficial organisms from Roundup Ready® canola trials in Australia or Europe or trials and commercial cultivation in Canada and the USA.

1.2.2 Invertebrates and insects

238. 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 any known toxicity of the introduced proteins and their low level of expression suggest that insect feeding on the plants will not unduly affect the ability of these insects to reproduce or function normally (USDA-APHIS 1999a).

239. Honey bees are a major pollinator of canola, and hives may be deployed in breeding, seed increase and general canola production (Manning & Boldand 2000; OGTR 2002; Gibbs & Muirhead 1998). 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 2002; Somerville 2001; HoneyBee Australis 2001; Goodman 2001; Somerville 1999). The applicant stated there was no detectable difference in the timing or length of the flowering period or pollen production (measured as seed yield) in Roundup Ready® canola GT73 compared to the non-GM variety (data provided by Monsanto).

240. Honeybees have been suggested to be a useful non-target arthropod species to assess the potential effects of transgenic plants (Brodsgaard et al. 2003). Two field studies in Canada assessed the impact of Roundup Ready® canola GT73 on larval survival, pupal weight and adult survival of honeybees (Huang unpublished). No statistically significant differences were found between the GM canola and non-GM canola.

241. A study with GM glyphosate tolerant soybeans expressing the CP4 EPSPS protein found no adverse impacts on green cloverworm (Morjan & Pedigo 2002). Another study found no difference in arthropod pests between Roundup Ready® and conventional soybeans (McPherson et al. 2003).

242. Assessments by other regulators and advisory bodies have all concluded that Roundup Ready® canola GT73 is unlikely to impact on other organisms (Canadian Food Inspection Agency 1995a; Canadian Food Inspection Agency 1995b; Canadian Food Inspection

Agency 1996a; Canadian Food Inspection Agency 1996c; Canadian Food Inspection Agency 1996b; European Scientific Committee on Plants 1998a; European Scientific Committee on Plants 1998b; USDA-APHIS 1998a; USDA-APHIS 1999b; USDA-APHIS 2002b; USDA-APHIS 2002a). In its assessment of Roundup Ready® canola GT73, the United States Department of Agriculture stated that glyphosate tolerant canola has not been shown to be harmful to beneficial insects or invertebrates in the USA or Canada (USDA-APHIS 1998b).

SECTION 2 EXPOSURE

243. A wide range of organisms will be exposed to Roundup Ready® canola GT73. However, the novel proteins are expressed at low levels and are naturally found in the environment.

SECTION 3 LIKELIHOOD OF THE TOXICITY OR ALLERGENICITY HAZARD OCCURRING

244. Knowledge of the mode of action of the CP4 EPSPS and GOXv247 enzymes, and the lack of known toxicity for the introduced proteins, suggests that it is highly unlikely that the deployment of Roundup Ready® canola GT73 will lead to deleterious effects on other organisms. An examination of information provided by the applicant, references from the literature and the conclusions of other regulatory assessments show that Roundup Ready® canola GT73 is unlikely to have any toxic effects on other organisms including animals (agricultural or native), insects or other invertebrates including microbes and soil biota when compared to non-GM canola.

245. The risk that Roundup Ready® canola will be more toxic to other organisms than conventional canola is negligible, as summarised below:

AGRICULTURAL OR NATIVE ANIMALS (VERTEBRATES)

- the CP4 EPSPS and GOXv247 proteins are not toxic;
- the introduced proteins occur naturally in soil organisms;
- these proteins are expressed at low levels in leaf and seed tissues; and
- feeding studies demonstrate that there are no anti-nutritional effects of the genetic modifications in the GM canola.

MICROBES AND SOIL BIOTA

- both of the introduced genes are derived from commonly occurring soil bacteria and the encoded proteins can be expected to already be present in soil;
- there are no reports of adverse effects of Roundup Ready® canola GT73 on invertebrates or microbial pathogens during trials in Australia or Europe or commercial production in North America;
- Roundup Ready® canola GT73 showed no observable differences in susceptibility to pathogens or insect pests in Australian and European field trials; and
- no significant differences have been detected in soil microbe populations between Roundup Ready® canola GT73 and conventional canola.

INVERTEBRATES AND INSECTS (ESPECIALLY HONEY BEES)

- the introduced proteins occur naturally in soil organisms;
- pollen production is normal in Roundup Ready® canola GT73;

- no differences between the larval survival, pupal weight and adult survival of bees foraging on the Roundup Ready ® canola GT73 or conventional canola; and
- a study with GM glyphosate tolerant soybeans expressing the CP4 EPSPS and GOXv247 proteins found no adverse impacts on green cloverworm or arthropod insects.

APPENDIX 4 ENVIRONMENTAL SAFETY - WEEDINESS

246. 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.

247. 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.

248. Weeds are plants that are considered pests either in managed ecosystems such as farms or in undisturbed habitats. Weed species typically spread easily in disturbed areas or within 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).

249. 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).

250. 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

251. Monsanto is seeking approval for the unrestricted commercial release in Australia of Roundup Ready® canola derived from transformation event GT73. Roundup Ready® canola is tolerant to glyphosate, the active constituent of the proprietary herbicide Roundup®.

252. Monsanto proposes the commercial cultivation of Roundup Ready® canola in all the current and future canola growing regions of Australia. Monsanto proposes a phased introduction to the market with the rate of increase in the area cultivated to Roundup Ready® canola being dependent on market acceptance, seed and variety availability. Roundup Ready® canola has been trialed previously in Australia under limited and controlled conditions.

253. This appendix investigates the potential for Roundup Ready® canola to be harmful to the environment due to increased potential for weediness. 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 the introduced herbicide tolerance trait or increased fecundity.

254. This assessment has adopted a cautious approach in considering whether the commercial scale release of Roundup Ready® canola without any specific containment or management conditions poses a risk of weediness impact on the environment.

255. Roundup Ready® canola contains two genes that confer tolerance to glyphosate. The *C4 EPSPS* gene from the bacterium *Agrobacterium* sp. strain CP4 and *goxv247* gene from the bacterium *Ochrobactrum anthropi.* The CP4 EPSPS protein is insensitive to inhibition of a key metabolic pathway by glyphosate and the GOX enzyme detoxifies glyphosate (see Appendix 1 for details).

256. Glyphosate is a broad-spectrum herbicide and is the active constituent of a range of proprietary herbicides registered by the APVMA, including Roundup® (APVMA 2003a). Glyphosate is registered for non-selective (general) weed control in broadacre agriculture, horticulture and non-cropped areas including industrial areas and roadsides and is a widely used chemical in these areas (Dignam 2001; Neve et al. 2003b, S. Powles, University of Western Australia, pers. comm).

257. Conventional canola is sensitive to glyphosate and this herbicide can be used for control of conventional canola as a volunteer weed.

258. In 2001 the APVMA registered glyphosate under the trade name 'Roundup Ready herbicide by Monsanto' for 'over the top' use for post-emergent (ie after the crop has germinated) weed control in Roundup Ready® cotton. The APVMA recently approved a parallel application to this one to vary the registration to extend the use for the same purpose on Roundup Ready® canola crops (APVMA 2003a; APVMA 2003b).

259. Although Roundup Ready® canola volunteers cannot be controlled by Roundup® or other glyphosate-based herbicides, they can still be controlled by alternative herbicides (Hall et al. 2000; Senior et al. 2002). Field observations by Monsanto confirm that Roundup Ready® canola is still susceptible to other herbicides that control related weedy species (eg. phenoxys and sulfonylureas).

260. Each herbicide is classified into a group depending on its mode of action, with each group having a different mode of action. Glyphosate is a group M herbicide and is the only group M herbicide registered by the APVMA in Australia.

261. Consideration of the changed use of herbicides if Roundup Ready® canola is adopted by industry and the potential for herbicide resistance developing in weed species as a consequence of herbicide usage is provided in Appendix 6.

SECTION 2 LIKELIHOOD OF THE WEEDINESS HAZARD OCCURRING

262. In assessing the likelihood of Roundup Ready® canola showing increased potential for weediness, the inherent weediness of conventional canola was assessed in a number of

ecosystems including agricultural, uncropped but disturbed habitats, and undisturbed natural habitats, and these traits compared to those of Roundup Ready® canola within those same systems.

Section 2.1 Inherent Weediness of Conventional canola

263. 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 primarily but not exclusively self-pollinating;
- outcrosses as a result of pollen tranfser by unspecialised pollinators or wind; and
- has high seed output under favourable conditions.

264. Although canola has a number of 'weedy traits', it is a poor competitor and does not establish well in undisturbed areas (Salisbury 2002d). Canola does occur in disturbed habitats along seed transport routes such as roadsides and railway lines, as well as field margins and wastelands in all countries where it is grown. However, it is not considered invasive in these habitats 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).

Agricultural systems

265. Canola can represent a 'volunteer' weed in cropping situations as a result of germination of spilt seed.

266. 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 prior to planting the next crop in the rotation is part of normal weed control operations and forms an integral part of agricultural production.

267. 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 losses often result in high densities of plants occurring as weeds ('volunteers') in subsequent crops (Legere et al. 2001).

268. 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 et al. 2003). With an average canola yield of 1.5t/ha in Australia and Canada, this would equate to 675-3,825 seeds/m^2 (Salisbury 2002d). Gulden et al.(2003) noted that incorrect harvester settings and excessive harvester speed can contribute to significant harvest losses, and that improved harvest management can reduce additions to the canola seedbank (Thomas 2000). Seed loss at harvest can also be reduced by windrowing at about 20-35% seed colour change to decrease shatter loss (Thomas 2000). The majority of Australian canola crops are windrowed to minimise seed loss through pod shatter at harvest (Walton et al. 1999).

269. Large numbers of viable conventional canola seeds to persist in the seedbank for several years (Lutman 1993; Pekrun et al. 1998).

270. At maturity, canola seed exhibits no primary 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 or sub-optimal oxygen in darkness (Lopez-Granados & Lutman 1998; Gulden et al. 2000; Pekrun et al. 1997b; Linder 1998; 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(Squire 1999; Pekrun et al. 1997a). The development of secondary dormancy can vary considerably between cultivars and even between seed lots from the same cultivar (Pekrun et al. 1997a; 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).

271. 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 differences 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.

272. It has been recommended to retain seed on the soil surface for as long as possible to avoid exposure to darkness and thus avoid the induction of secondary dormancy and seed persistence in the soil (Lopez-Granados & Lutman 1998, C. Preston pers. comm.). Light sensitivity can develop in canola enabling the seed to germinate in response to very short exposure to light, as experienced during soil cultivation. Therefore in low tillage situations, where large quantities of crop residue create shaded conditions (Legere et al. 2001) may result in greater seed dormancy.

273. 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 amenable to the development of secondary dormancy than in the Northern hemisphere where conditions after harvest are cool and moist (J. Baker, CRC for Australian Weed Management, pers. comm.). 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.

274. 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 of volunteers (0.2 to 0.5 plants/m^2) present in fields 4 to 5 years after a canola crop (Legere et al. 2001; Simard et al. 2002). The size of the seedbank can be minimised if measures are taken to reduce it. It is noteworthy that seasonal variation in seedbank density in Canada occurred as a result of additional seeds being produced by volunteer plants each spring thereby replenishing the seedbank (Legere et al. 2001).

Uncropped disturbed habitats

NORTHERN HEMISPHERE

275. Canola is not considered an invasive weed and its dissemination normally results from seed spillage during harvest and transport operations.

276. Persistence of canola seed is considerably longer in uncultivated 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 period of time may also suggest that the presence of canola in these locations was not considered a problem and that the canola was not subject to active management.

277. 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 these feral canola populations are self-sustaining (Wilkinson et al. 1995).

278. 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 seeds by trucks (Crawley & Brown 1995).

279. A recent analysis of 41 feral canola populations in France examined whether there is a significant seedbank associated with these populations. Their results confirmed the existence of a small seedbank. They concluded that the majority of seeds germinate or die in the first year, and that external seed flow from nearby crops or seed transport and population self-recruitment are very important in the maintenance of feral populations (2003).

AUSTRALIA

280. A survey for the presence of canola plants along 4000km of representative roadsides in the major canola growing regions of Australia, including New South Wales, Victoria, Western Australia, South Australia and Tasmania was conducted in September/November 2001 (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 10-km in an area 20 m by 5 m (100 m^2). 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. In the majority of cases, canola was only found within the first 5 m from the roadside and not beyond, indicating that initial spread of seed was from transport along roads.

281. The frequency of roadside canola was re-assessed in the canola growing regions of Victoria in September 2002 (Norton 2003a; Norton 2002). The survey found roadside

densities of canola ranged between 3.8 – 100.1 plants/km. There were a number of 'hot spots' recorded (ie. densities greater than 50 plants/100m) along some roadsides. These 'hot spots' were observed on road bends or channels and likely to have been a result of seed spillage from uncovered trucks in short transit to receival points. These results confirmed canola has limited potential to spread and invade in Australian conditions. The majority of canola plants were found between 1 and 2 metres from the roadside, in disturbed areas of bare soil/roadbase or roadbase/bluemetal mixture adjacent to the bitumen.

282. Other recent studies in Australia have demonstrated that feral canola populations have high extinction rates, often failing to establish and persist as new generations. Studies conducted in South Australia in 2002 showed that over a 2 year period, for a number of canola populations deliberately planted in roadside habitats, only a single seedling survived to set seed and no seedlings emerged in the following year (J. Baker, pers. comm.). These results suggest that while feral populations do germinate along roadsides, they rarely establish and persist.

283. These results also demonstrate that canola is a poor competitor in disturbed uncropped habitats, such as roadsides, particularly in the presence of other weeds (J. Baker pers. comm.). The number of canola plants found on grassy verges is often low which indicates that canola is not a strong competitor where other plants are present (Norton 2003a).

284. Dignam (2001) conducted a survey of the prevalence of canola as a weed in non-agricultural habitats throughout Australia. The survey concentrated on canola growing areas, and data was collected by interviewing council, road and rail authorities, and National Park weed personnel.

285. The survey data also support the conclusion that canola is an insignificant weed in these areas. Canola was reported by only 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 as a weed, approximately 70 % did nothing to control it. However, where canola was controlled glyphosate was reported as the main method used. Management of roadside canola is readily achievable due to its transient nature and the availability of a number of management strategies, including a range of herbicide options or mowing.

286. The survey results also indicated that no weed control operations were undertaken in significant proportions of the areas managed by respondents: 59 % of council lands, 56% for road and rail and 93 % of National Parks.

Undisturbed natural habitats

287. There are no studies which provide evidence that canola is a significant or invasive weed of natural ecosystems, neither in Canada (Beckie et al. 2001; Canadian Food Inspection Agency 1994; Warwick & Small 1999) nor Australia (Salisbury 2002d). 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 U.K. study of 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 mentioned 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 Roundup Ready® canola

288. There is no evidence to suggest that the genetic modifications and introduction of the CP4 EPSPS and *goxv247* genes have resulted in phenotypic traits that would cause Roundup Ready® canola to be more weedy than conventional canola. Roundup Ready canola is not tolerant to herbicides other than glyphosate (Senior et al. 2002). Herbicide tolerance will not confer any advantage to volunteer glyphosate tolerant canola outside the system where the herbicide is used. The genetic modification and expression of the respective genes are described in detail in Appendix 1.

289. Studies conducted in Canada (Warwick et al. 1999; Agriculture and Agri-Food Canada (AAFC) 1995), Britain (Norris et al. 1999) and the United States (USDA-APHIS 1998b), have not found any evidence that the herbicide tolerance trait has made Roundup Ready® canola more intrinsically weedy.

290. The growth characteristics of Roundup Ready® canola in terms of phenology (eg flowering period); pollen production and viability; seed production, germination and dormancy; and agronomic performance, including disease susceptibility are not substantially different to conventional canola varieties (informationsupplied by Monsanto, Manitoba Agriculture and Food 2003). Seed pod shattering, and seed size and weight of Roundup Ready canola does not differ from conventional canola indicating that seed characteristics have not altered (Nickson et al. 1994, Monsanto Unpublished; Nickson and Taylor 1994, Monsanto Unpublished; Agriculture and Agri-Food Canada (AAFC) 1995; Taylor 1995, Monsanto Unpublished; Monsanto Company 2002). Roundup Ready® GT73 canola has been developed using elite Australian breeding lines and therefore any growth and agronomic characteristics will be within the range of conventionally developed canola cultivars.

Persistence of Roundup Ready® canola

291. Herbicide tolerance is unlikely to confer any advantage to volunteer glyphosate resistant canola and/or weedy relatives outside of the system where the herbicide is used. There is also no evidence to suggest that the genetic modifications to Roundup Ready® canola have resulted in pleiotropic traits that would increase its weediness.

292. In U.K. trials, the number of glyphosate tolerant canola volunteers in the year following a GM canola crop were comparable to or less than conventional canola (Crawley et al. 1993; Norris et al. 1999; Sweet & Shepperson 1998; Sweet 1999). Information from commercial fields in Canada show the same trend (Derksen et al. 1999; MacDonald & Kuntz 2000). The incidence of Roundup Ready® canola volunteers recorded in Monsanto and OGTR monitoring reports at Australian release sites is consistent with the incidence of volunteers in the U.K and Canada, measuring from zero to several thousand (Norris et al. 1999; MacDonald & Kuntz 2000; Salisbury 2002d).

293. The capacity for large numbers of viable conventional canola seeds to persist in the seedbank for several years (Lutman 1993; Pekrun et al. 1998) appears to apply equally to glyphosate tolerant canola. Large numbers of glyphosate tolerant canola seeds persisted in the soil for up to three years after their release at some U.K. sites (Norris et al. 1999). Canadian data show Roundup Ready® canola volunteers can persist for at least 3 years after a crop (Simard et al. 2002) and similar results were obtained from trials in Denmark (Fredshavn & Poulsen 1996).

294. Salisbury (2002a) has noted that the incidence (germination rates) of volunteers at sites from previous Australian trials of GM canola (glufosinate ammonium and glyphosate 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 pers. comm.). In the U.K., the number of glyphosate 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).

295. Analysis of monitoring reports from Australian GM 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 2002d). Recent reports from OGTR monitoring indicate that the management practices and use of the sites after harvest of canola also affect persistence (eg burial of seed as a result of deep cultivation after harvest).

Other Attributes

296. There are no measurable differences in the ability of Roundup Ready® canola plants to adapt to biotic or abiotic stress factors. The applicant has indicated that Roundup Ready® canola does not show any change in resistance or susceptibility to major canola pests and pathogens (such as *Leptosphaeria maculans* which causes blackleg disease). Monsanto has indicated that results of field trials in Australia and commercial releases in other countries show no differences between Roundup Ready® canola and conventional canola in ability to resist drought, heat and frost.

297. Canola or *Brassica juncea* are sometimes sown for biofumigation purposes. 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 & Sarwar 1998). Glyphosate tolerance does not have any effect on the biofumigation properties of Roundup Ready® canola (J. Pratley and R. Stanton, Charles Sturt University, pers. comm.).

Commercial production of Roundup Ready® canola in Canada

298. Roundup Ready® canola varieties have been grown commercially in Canada since 1995 (Agriculture and Agri-Food Canada (AAFC) 1995). The uptake of herbicide tolerant canola in Canada has been rapid, with GM varieties accounting for a significant proportion of the canola crop in recent years(Harker et al. 2002; Serecon Management Consulting Inc & Koch Paul Associates 2001; Beckie et al. 2001).

299. Approximately 50 % of the area sown to canola in Western Canada in 2002 was Roundup Ready® canola. Only 15% was non-herbicide tolerant canola and the remaining area was sown to other herbicide tolerant (GM and non-GM) canola cultivars (R. Van Acker, University of Manitoba, pers. comm.). Volunteer canola represents a weed of agricultural production systems in Canada (Martens 2001; Beckie et al. 2001; Legere et al. 2001; Simard & Legere 2001; Simard et al. 2002). For example, in Manitoba in 1997 it was ranked the 19th most abundant weed (Martens 2001), and 4th in the central region of Manitoba in 2001 (Kaminski 2001). Herbicide tolerant volunteer canola has been singled out by some respondents in surveys (Martens 2001; Kaminski 2001). These concerns have also been mirrored in reports in the popular press (eg Ewins 2001) and anecdotes from farmers and agronomists have suggested that volunteer canola has increased in weediness due to the herbicide tolerance traits (Martens 2001).

300. However, as noted above there is no indication that Roundup Ready® canola is more intrinsically persistent than conventional canola (Derksen et al. 1999; MacDonald & Kuntz

2000). Gulden et al (2000) found no significant differences between dormancy of Roundup Ready® canola, or other herbicide tolerant canola and conventional canola, but did find significant differences between varieties, indicating that the parental genotype is an important factor in the degree of dormancy (Gulden et al. 2002).

301. Issues of volunteer control *per se* (Gulden et al. 2002) and gene flow (Hall et al. 2000) have contributed to the apparent increase in the incidence of volunteer canola observed in Canada since the widespread introduction of herbicide tolerant varieties.

302. Prior to the introduction of herbicide tolerant varieties, canola volunteers in subsequent seasons did not represent a significant issue because they would be completely controlled by non-selective herbicide application.

303. In contrast, in situations where a herbicide tolerant variety has been grown the persistence of volunteers in subsequent seasons will become apparent if the same herbicide is used for weed control because these volunteers will escape control. This has particularly been the case where glyphosate is relied on for weed control in minimum tillage situations (Derksen et al. 1999). Glyphosate has provided ineffective weed control because of Roundup Ready® canola volunteers, even several years after the canola crop.

304. The education of farmers in Canada is now generally considered to have been inadequate with regard to the introduction of herbicide tolerant varieties, particularly the short and long term implications for volunteer control (Simard et al. 2002; Entz & Martens 2003b; Entz & Martens 2003a).

305. 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 widespread introduction of herbicide tolerant varieties has meant that control of volunteer canola is more complex (Derksen et al. 1999), and highlighted the persistence of canola volunteers in the seedbank for several seasons and the capacity for pollen flow between crops that have always been present in the cropping system (Martens 2001).

306. Lack of awareness of this information led to growers being surprised when volunteers in paddocks neighbouring herbicide tolerant canola showed resistance to that herbicide (Simard et al. 2002), even though they could be readily controlled by the application of alternative herbicides (Beckie et al. 2001).

307. Although the incidence of volunteers normally declines in successive seasons, volunteers may still be present even after 4 years (Legere et al. 2001; Simard et al. 2002), and Gulden et al (2002) have recommended a separation of at least 4 years between subsequent canola crops, and that these should be of different herbicide tolerance characteristics. Failure to manage the canola seedbank to reduce numbers, for example by allowing volunteers in early seasons to flower and replenish the seedbank, would further exacerbate this situation (Legere et al. 2001).

308. In addition, there have been reports of glyphosate-tolerant canola volunteers in fields previously sown to canola, but where Roundup Ready® canola had not been sown (Hall et al. 2000; Beckie et al. 2001). As Roundup Ready® canola was grown in adjacent fields, these instances have been attributed to pollen flow, however other studies have identified the presence of herbicide tolerant varieties in commercial lots of conventional seed which may have been the cause of the volunteers (Downey & Beckie 2002; Friesen et al. 2003).

309. Entz and Martens (Entz & Martens 2003b; Entz & Martens 2003a) have reported that failure to implement appropriate management strategies has led to a situation in Canada where canola volunteers tolerant to all three herbicide systems (glyphosate, glufosinate

ammonium and imidizolinone tolerant) are present in many fields where canola has been grown. They also note that the containment of Roundup Ready® canola volunteers has been very difficult, due in large part to the heavy reliance upon glyphosate in western Canadian farming systems (Entz & Martens 2003a).

310. 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.

311. It should also be noted that the number of glyphosate tolerant canola volunteers appearing in neighbouring fields as a result of gene flow will be minimal compared to those occurring in the field following the harvest of the Roundup Ready® canola crop. (Appendix 5 deals with gene flow and multiple herbicide resistant volunteers in detail.)

Roundup Ready® canola in the Australian cropping system

312. As described above, there is no evidence to suggest that Roundup Ready® canola is intrinsically more invasive or persistent than conventional canola, either in Australia or overseas and in terms of weediness, the main impact of Roundup Ready® canola in the agricultural environment will be as a volunteer in subsequent seasons.

313. As with conventional canola volunteers, the management of Roundup Ready® canola volunteers will present an agricultural production issue with a potential economic impact in terms of alternative weed management choices, but will pose no greater risks to human health and safety or the environment than conventional canola.

314. Roundup Ready® canola volunteers would obviously not be controlled by glyphosate. However, Roundup Ready® canola can be managed and controlled using a variety of alternative herbicides (see below for further details) and non-chemical management techniques currently used to control conventional canola.

315. It is important to note that the main implications for volunteer control would arise from the choice of individual farmers to grow Roundup Ready® canola, and that they apply predominantly to the paddocks in which it is sown. (Control of glyphosate tolerant volunteers resulting from gene transfer is addressed in Appendix 5).

316. Management of Roundup Ready® canola persistence can be achieved through the application of the already established principles and practices of integrated weed management: 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.

317. To avoid the problem of persistence of either conventional or Roundup Ready® canola volunteers in subsequent seasons, good crop husbandry is required to minimise crop losses at harvest. Seed loss at harvest has been reported at between 1.5 and 8.5% (CETIOM 2000) and can be reduced by windrowing when 20 to 35% of seed has changed colour, using properly adjusted machinery and reducing combine speed (Thomas 2000).

318. 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).

319. Management choices immediately post-harvest will have a critical impact on the incidence and persistence of volunteers in subsequent generations. To prevent seed burial and induction of secondary dormancy giving rise to a persistent seedbank, seed should be left on the surface for as long possible and deep cultivation avoided. Appropriate levels of soil disturbance can also provide a pro-active management tool for reducing the seedbank by stimulating buried seeds to germinate and emerge. In Canada, seedbanks of GM glyphosate tolerant canola have been managed by delaying cultivation (leaving seed on the soil surface) and a shallow soil cultivation (Pekrun et al. 1998; Thomas 2000). Volunteers in subsequent crops can be controlled with an appropriate registered herbicide or in non-crop situations by preventing the plants from reaching maturity by mowing, grading or herbicide application.

320. Glyphosate can be used in combination with some herbicides, eg by tank mixing, which gives the flexibility to apply a herbicide treatment in a situation where there is a mixed weed spectrum, including glyphosate tolerant volunteers and other glyphosate-susceptible brassicaceous weeds. Such tank mixing or 'spiking' strategies have also been adopted for other glyphosate tolerant crops (Ellis & Griffin 2003; eg Roundup Ready soybean or corn in the USA, Gonzini et al. 1999; Hahn & Stachowski 2002) and in other situations where enhanced knockdown of difficult to control weeds is required (Howey 2002; Davies 2002; Goldwasser et al. 2003; Cumming 2002).

321. Table 2 shows the herbicide options that can be used to control Brassica weeds, including canola volunteers, in a range of cropping situations. It should be noted that a range of other non-chemical options are available for the control of Brassica weeds and canola volunteers, including slashing and mowing, green manuring, cultivation and rotational choices, eg the dense growth habit of some cereal crops provides an effective means of suppressing canola volunteers.

Table 2 Herbicides for control of Brassica weeds in crop and fallow.

Herbicide	Group	Rate/ha	Situation	Tank-Mix with Glyphosate
Chlorosulfuron	B (ALS inhibitor)	15-20g/ha	Wheat, (barley and oats post emergence)	Yes
Metsulfuron	B (ALS inhibitor)	5-7 g/ha	wheat, triticale, barley, fallow	Yes
Metsulfuron + thifensulfuron	B (ALS inhibitor)	30-35g/ha	wheat, barley	No, in-crop only
Flumetsulam	B (ALS inhibitor)	15-25g/ha	wheat, barley, oats, lupins	Possible, but not practised
Triasulfuron	B (ALS inhibitor)	30-35g/ha	wheat (pre only)	Yes
Tribenuron	B (ALS inhibitor)	20-25g/ha	Fallow	
Metosulam	B (ALS inhibitor)	5-7g/ha	wheat, barley, oats, lupins	Possible, but not practised
Imazamethapyr	B (ALS)	0.2-0.3L/ha	field pea, faba bean	Yes
Triasulfuron + terbutryn	B (ALS inhibitor) + C	250-500g/ha	wheat, barley	No, in-crop only
Cyanazine	C (triazine)	3 or 4L/ha	chickpea, field pea, faba bean	Yes
Metribuzin	C (triazine)	0.435-0.58L/ha	chickpea, field pea, faba bean	No

Herbicide	Group	Rate/ha	Situation	Tank-Mix with Glyphosate
Simazine + prometryn	C (triazine)	1.5+1.5L/ha	Chickpea	No
Terbutryn	C (triazine)	0.85-1.1L/ha	Oats	No, in-crop only
Simazine	C (triazine)	0.8-2L/ha	lupins, chickpea, faba beans, lentil, TT canola	Yes
Atrazine	C (triazine)		TT canola. Sorghum, maize, fallow	Yes
Simazine + imazathepyr	C (triazine) + B (ALS)		Chickpea	Yes
Simazine + diflufenican	C (triazine) + F		Lupins	No, in-crop only
Diflufenican	F (Inhibitors of carotenoid biosynthesis)	0.15-0.2L/ha	field pea, lupins	No, in-crop only
Diflufenican+ MCPA	F (Inhibitor of carotenoid synthesis)+I	0.5-1.0	wheat, barley, oats	No, in-crop only
Diflufenican + bromoxynil	F + C	0.5-1.0	wheat, barley	No, in-crop only
2,4-D amine	I (phenoxy)	0.7-2.1L/ha	wheat, barley, oats, fallow	No
2,4-D IPA	I (phenoxy)	0.8-1.6L/ha	Fallow	Yes
2,4-D ester	I (phenoxy)	0.35-0.7L/ha	wheat, barley, fallow	Yes
MCPA amine	I (phenoxy)	0.35-1.6L/ha	wheat, barley, oats, field pea	No
MCPA LVE	I (phenoxy)	0.5-1.6L/ha	wheat, barley, oats	Yes
2,4-DB	I (phenoxy)	2.1-3.2L/ha	wheat, barley, oats, lucerne. Medics	No
Diuron	I (urea)	0.9L/ha	Oats	No, in-crop only
Diuron+MCPA	I (urea) + I (phenoxy)	0.28 + 0.5	wheat, barley	Yes
Paraquat + diquat	L (bipyridil)	1.6-2.4L/ha	Fallow	No

322. It should be noted that the APVMA is currently conducting a review of the registration of 2,4-D (APVMA 2003c).

323. Glyphosate is also used extensively in vineyards to control a wide range of broadleaf and grass weeds. Canola or *Brassica juncea* are sometimes sown in vineyards for biofumigation. Roundup Ready® canola would not be controlled by glyphosate. While this would not present a risk to human health and safety or the environment, Monsanto's *Roundup Ready® Canola Crop Management Plan* (RRCMP) states that Roundup Ready® canola must not be grown in vineyards for biofumigation purposes (Monsanto Australia Ltd 2002). Table 3 shows the herbicide options that can be used to control *Brassica* weeds, including Roundup Ready® canola volunteers, in vineyards.

Table 3 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®
Glufosinate ammonium	N	Basta®

324. As glyphosate is so widely used in Australian agriculture, especially in minimum and zero tillage operations, the commercial release and adoption of Roundup Ready® canola in the Australian cropping system would have on-farm implications for the choice of herbicides for weed control operations in subsequent crops. In particular it would involve modification of current weed control strategies in the broadacre cropping rotation, especially the high reliance on glyphosate for pre-sowing weed knockdown.

325. Reliance on non-selective herbicides, especially reliance on one herbicide, increases the likelihood of development of resistant weeds because the repeated use of a herbicide over time will gradually select for those plants within the population which have a naturally higher tolerance to the herbicide. The risk of herbicide resistance arising as a consequence of herbicide usage is considered in Appendix 6.

Monsanto's Crop Management documents

326. As noted above, volunteer control issues associated with the introduction of herbicide tolerant canola varieties in Canada, including Roundup Ready® canola, may have been exacerbated because of inadequate education of producers.

327. Monsanto has developed a package of documents to support the management of the introduction of Roundup Ready® Canola in Australia: *Roundup Ready® Canola Crop Management Plan* (RRCMP*), Roundup Ready® Canola Technical Manual* (RRTM) and *Roundup Ready® Canola Resistance Management Plan* (RRRMP). These plans recommend that farmers pay particular attention to volunteer management and herbicide selection in order to effectively control canola volunteers. To minimise the canola seed bank they recommend that growers:

- clean machinery and trucks to reduce spread of GM seed;
- assess fields and adjacent areas for the presence of volunteers and choose appropriate management techniques (eg herbicides, grazing or cultivation) to remove volunteers prior to flowering and seeding;
- use crop rotations that allow removal of volunteers; and
- keep accurate field records.

328. Draft versions of these documents have been declared as Confidential Commercial Information (CCI) under section 185 of the Act**.** The company has subsequently developed the documentation further but they cannot be finalised until regulatory approvals are

received from the Regulator and the APVMA. However Monsanto have indicated that the final versions of these documents will be made publicly available as soon as possible, if and when the release of Roundup Ready® canola is approved by the Regulator and 'Roundup Ready herbicide by Monsanto' is registered by the APVMA, and the relevant regulatory requirements have been incorporated.

329. Monsanto's RRCMP recommends that any canola volunteers present in a paddock where Roundup Ready® canola has been grown in the previous 3 years should be controlled, and that in minimum tillage situations, knockdown herbicides (with an appropriate tank mix if using glyphosate-based products) should be used and combined with a light cultivation where appropriate in conventional tillage situations. The RRRMP recommends that glyphosate not be used in the year following harvest of Roundup Ready® canola, bearing in mind Roundup Ready® canola volunteers would not be controlled by glyphosate.

330. To minimise the spread of glyphosate tolerant canola seed, it is recommended that equipment be cleaned between seeding or harvesting operations, all genetically modified Roundup Ready® seed should be stored separately and be clearly labelled, and spillage during transport should be avoided as far as possible.

331. Alternative herbicide options can effectively manage conventional and Roundup Ready® volunteer canola. In situations where glyphosate-based products are used, Monsanto recommend a tank mix with 2,4-D or MCPA. (However, it should be noted that while 2,4-D is currently registered in Australia that the APVMA is currently conducting a review of its registration (APVMA 2003c)).

332. However, herbicides are not the only tools to manage conventional and Roundup Ready® volunteer canola. The *Roundup Ready® Canola Crop Management Plan* makes a number of recommendations to growers to prevent persistence and spread of Roundup Ready® canola. They recommend growers consider adopting a diverse range of management strategies including cultural practices, such as cultivation, slashing, burning, crop competition or grazing to control emerged plants and shallow soil disturbance to stimulate buried seeds to germinate.

Conclusion on Roundup Ready® canola in agricultural systems

333. Roundup Ready® canola will be no more invasive or persistent than conventional canola. The likelihood that Roundup Ready® canola will persist in agricultural production systems as a volunteer weed is the same as for conventional canola.

334. Roundup Ready® canola will not be controlled by glyphosate, but it can be readily controlled by a variety of herbicide and non-chemical management practices currently used to control volunteer canola. Growing Roundup Ready® canola would have implications for the choice of herbicide(s) used for subsequent weed control operations on-farm.

335. The risk of Roundup Ready® canola resulting in adverse impacts on the environment as a result of weediness in agricultural systems is therefore considered to be negligible, and no specific licence conditions are proposed for this release.

Dissemination of seed by animals

336. The possible dissemination of Roundup Ready® canola seed by animals or birds, either within the agricultural situation or to other habitats, has been considered.

337. It is conceivable that small amounts of seed could be dispersed in the faeces of grazing livestock.

338. An Australian study found that viable canola seed was excreted from sheep for up to 5 days after it was included in the diet (Stanton et al. 2003b). The percentage of viable 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 fifth 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.

339. To prevent the possible dispersal of viable glyphosate tolerant canola seed in the faeces of stock grazing on Roundup Ready® canola stubble, Monsanto's RRCCMP recommends that livestock be held within a single grazing area for a period of at least 7 days.

340. 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 and remove pods during development and at maturity (Stanley & Marcroft 1999). While no direct experimental data is available to assess the likelihood of dispersal of viable canola seed by wild birds, canola is soft-seeded and is very unlikely to survive passage through the gut of a bird. There is no evidence that Roundup Ready® canola is more likely to be consumed by birds than conventional canola.

341. Canola seed may be used in poultry feed. Growers in some areas of Australia apply poultry manure from poultry farming operations to fields as fertiliser, however no incidences of volunteer canola weed problems resulting from the application of manure have been reported.

342. As noted previously, seed shattering ability, seed size and seed weight of Roundup Ready® canola is no different to conventional canola, indicating no alteration in the potential for seed dispersal as a result of the genetic modifications. 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 mechanism for the dissemination of Roundup Ready® canola.

343. Data from Australia, Europe and North America support the conclusion that the primary means of dispersal of canola are via human activities such as sowing, harvesting and transport, and handling pre- and post-harvest. Even if Roundup Ready® canola seed were to be disseminated by livestock or native animals within the agricultural system it would present the same management problem as the volunteers. The likelihood that Roundup Ready® canola might establish and persist in undisturbed habitats is considered negligible because canola is not invasive, is a poor competitor and is not considered a weed of undisturbed habitats. Similarly the risk that the dissemination of Roundup Ready® canola by livestock or native animals will result in adverse impacts on the environment is considered to be negligible.

Uncropped disturbed habitats

344. Due to its primary colonising nature, canola can take advantage of disturbed land (Salisbury 2002d), 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) 1997; 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; Senior & Dale 2002) except in the presence of the specific herbicide. Furthermore, Roundup Ready® canola is no more fit than conventional canola and does not have traits that confer enhanced fitness such as enhanced stress adaptation, other than tolerance to the herbicide glyphosate (Agriculture and Agri-Food Canada (AAFC) 1995).

345. Monitoring results from Canada of unmanaged areas adjacent to fields and along transportation corridors indicate that the frequency of GM herbicide tolerant canola volunteers is comparable to that of 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). 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 generally due to plants being missed during control operations (Wilkinson et al. 1995). In a recent roadside survey for volunteer canola in Australia, Norton (2003a; 2002) observed that some large canola plants were found adjacent to guide posts, apparently protects from control by mowing operations.

346. As previously noted, recent roadside surveys in the major canola growing regions of Australia found that in most cases canola plants were growing within 5 m of the roadsides (Agrisearch 2001; Norton 2003a), with some plants being 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. Of those reporting canola, approximately 70 % did nothing to control it (Agrisearch 2001).

347. Of the authorities that do actively manage weeds in these habitats, in and around canola growing areas throughout Australia accounts for 75 % and 65 % of chemicals used by Road and Rail authorities and Local Councils respectively (Dignam 2001).

348. Where glyphosate is the chosen herbicide for control of weeds along transport routes (road verges, railway lines, grain depots etc.), field edges, wastelands or fencelines, the survival and persistence of feral glyphosate tolerant canola plants would be enhanced. In situations where glyphosate has been relied on for the control of volunteer canola, the presence of Roundup Ready® canola would have implications for the choice of herbicide(s) selected for control operations.

349. It should be noted however, that although glyphosate is a broad spectrum herbicide, it would not be the herbicide of choice for the control of all weeds, as its effectiveness may be limited by environmental factors, the weed species present and the developmental stage of the weeds (Adkins et al. 1998; Brown et al. 2003a; Ellis & Griffin 2003). Glyphosate is generally considered to be most effective against grasses but may be less effective against some established broadleaf species (DPIWE Tasmania 2002; eg Shaw & Arnold 2002).

350. A report by Agriculture Western Australia (Anon. 2001) states that shire councils rely on herbicide mixtures to effectively control roadside weeds. Herbicide mixtures including a selective residual herbicide (eg triazine), or an inexpensive auxinic herbicide, such as 2,4-D or MCPA, would readily control the Roundup Ready® component of volunteer canola (Anon. 2001; Hall et al. 2000). Slashing is commonly used along roadsides as a fire reduction strategy, but has added weed control benefits, particularly where feral Roundup Ready® canola populations may exist.

351. Canola is found in low densities in disturbed non-cropped situations, such as grassy road verges (MacDonald & Kuntz 2000; Norton 2003a), has poor competitive ability, recruitment and spread where other plants are present (J Baker, pers. comm.), and tends to be transient in these environments. The available evidence supports the conclusion that Roundup Ready® canola would not pose a risk of adverse impacts in non-cropped disturbed habitats and poses no greater weed threat than conventional canola in these environments.

352. For non-crop situations on-farm, such as fencelines, roadsides and around sheds, Monsanto recommends that growers control canola prior to reaching maturity by mowing, grading or herbicide application as appropriate for the situation.

353. Although glyphosate is the predominant herbicide chosen for weed control by councils and road and rail authorities, management of Roundup Ready® canola in roadsides and other disturbed habitats would be readily achieved by the variety of the management strategies available, including a range of alternative herbicides to glyphosate, tank mixing of other herbicides with glyphosate, and non-chemical management methods such as mowing, cultivation, burning and grazing.

354. It should also be noted that reliance on a single herbicide for weed control operations is inconsistent with the principles of integrated weed management (Ensbey 2001; Buhler 2002) and is undesirable because it can result in the evolution of herbicide resistance (Gressel 2002; Llewellyn et al. 2001). Further consideration of the evolution of herbicide resistance through the overuse of herbicides is provided in Appendix 6. Monsanto have prepared a 'Tech Topic' on integrated weed management, including management of Roundup Ready® canola volunteers, in non-crop situations (Monsanto Australia Ltd 2003).

Undisturbed natural habitats

355. 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 do not generally prevail in non-cultivated areas and natural habitats. Genetically modified herbicide tolerant canola has no altered invasive capacity which would enhance its weedy potential in natural habitats (Canadian Food Inspection Agency 1995b; Rasche & Gadsby 1997; MacDonald & Kuntz 2000; Agriculture and Agri-Food Canada (AAFC) 1995; Norris et al. 1999). Glyphosate tolerant canola is not more competitive than its counterparts in natural habitats and its impact on biodiversity is equivalent to conventional canola (Agriculture and Agri-Food Canada (AAFC) 1995).

356. The potential weediness of GM herbicide tolerant canola in undisturbed habitats 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). The study examined glufosinate ammonium tolerant canola. Sites were monitored annually to follow the fate of sown individuals, to measure recruitment into 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 modifications to herbicide tolerant canola did not appear to result in weedy characteristics as no population of canola, conventional or GM, persisted beyond the second year. None of the crops increased in abundance at any of the sites. The results showed that GM herbicide tolerant canola was no more invasive or persistent than its conventional counterpart in situations where herbicides are not applied.

357. While no similar long term study has been conducted for glyphosate tolerant canola, either elsewhere overseas or under Australian conditions, the results of Crawley (1993; 2001) support the conclusion that the introduction of the glyphosate tolerance trait would

not be expected to affect the weediness of Roundup Ready® canola. Therefore Roundup Ready® canola would not be expected to be any more invasive or persistent than conventional canola in undisturbed habitats in Australia, and in a survey of National Parks in the major canola growing regions of Australia, canola was not reported as occurring as a weed by any weed personnel (Dignam 2001).

358. Roundup Ready® canola would only have a selective advantage in situations where it is exposed to glyphosate. However, it should be noted that broadcast spraying of vegetation does not occur in undisturbed habitats such as National Parks, and weeds are removed by spot spraying.

359. Where herbicides are used to control weeds in undisturbed environments glyphosate is frequently used, but removal is normally by spot spraying, not broadcast spraying. For control of other weeds in National Parks, 66 % of all chemicals used were glyphosate based. However, the survey results also indicate that 93% of National Parks are not subjected to weed management operations. It is therefore unlikely even if Roundup Ready® canola were to be present in an undisturbed habitat it would be exposed to glyphosate.

360. All available evidence supports the conclusion that canola is not a weed of undisturbed natural habitats, either in Australia or overseas, because it is not invasive and is a poor competitor. The introduction of herbicide tolerance traits to canola has not increased its invasiveness or competitiveness. The likelihood of Roundup Ready® canola establishing in undisturbed habitats is considered to be negligible. Even if this occurred it would be unlikely to persist because of its poor competitiveness and could readily be controlled with a variety of alternative herbicides and non-chemical management techniques. The risk of Roundup Ready® canola establishing as a weed in undisturbed habitats and resulting in adverse impacts on the environment is considered to be negligible.

SECTION 3 CONCLUSIONS REGARDING WEEDINESS

361. 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.

362. The introduced genes do not increase the potential weediness of the Roundup Ready® canola or provide these plants with an ecological advantage over conventional canola except in the presence of glyphosate. The germination, seed dormancy and fitness traits including herbicide sensitivity (except for glyphosate), disease resistance, insect susceptibility, stress adaptation and competitiveness are all within the range of conventionally bred canola varieties.

363. Roundup Ready® canola does not have any competitive advantage in the absence of glyphosate and its susceptibility to other herbicides is no different to conventional canola.

364. The APVMA has recently extended the registration of glyphosate as 'Roundup Ready® herbicide by Monsanto' for use 'in crop' on Roundup Ready® canola to control weeds.

365. Roundup Ready® canola can be managed and controlled using a variety of alternative herbicides and non-chemical management techniques currently used to control conventional canola.

In summary:

- The risk that in the absence of glyphosate Roundup Ready® canola will be more persistent or invasive in the agricultural environment than conventional (non-GM) canola and result in a more detrimental environmental impact is negligible;
- The risk that Roundup Ready® canola will be more persistent or invasive in non-cropped disturbed environments than conventional (non-GM) canola and result in a more detrimental environmental impact is negligible;
- Although Roundup Ready® canola will not be controlled by the application of glyphosate, it can be readily controlled by a variety of herbicides and nonchemical management practices currently used to control conventional canola.
- The removal of Roundup Ready® canola volunteers in agricultural or disturbed habitats will require a changed weed management strategy where glyphosate is the only method used.
- As with conventional canola volunteers, Roundup Ready® canola volunteers will present an agricultural production issue with a potential economic impact in terms of alternative weed management choices, but will pose no greater risks to human health and safety or the environment than conventional canola.
- The risk that Roundup Ready® canola will be more invasive or persistent in undisturbed environments than conventional (non-GM) canola and result in a more detrimental environmental impact is negligible;
- As the risk that Roundup Ready® canola will result in adverse impacts on the environment as a result of weediness is considered negligible, no specific licence conditions with respect to potential weediness are proposed for this release.

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

366. 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 by the proposed release. In this context, the potential for gene transfer from the GMO to other organisms was considered.

367. When analysing the risk of gene transfer (gene flow), 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 which may exhibit altered characteristics to either of their parents eg agronomic performance or fertility. Progeny derived from crosses between plants of different species or genera are known as inter-specific hybrids (often simply referred to as hybrids). Introgression is the incorporation of a gene or genes into the population (ie subsequent generations) after a hybridisation event.

368. Where gene transfer has the potential to cause an environmental problem the production of hybrids would represent a short term risk whereas any impacts related to introgression would involve a longer time frame.

369. A number of factors influence the likelihood of hybridisation 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 and hybrid viability.

370. Additionally, introgression would then require hybrid fertility, hybrid viability and fertility of progeny through several generations of backcrossing resulting in successful incorporation of the modified genes into the population. For successful introgression to occur all pre- and post-fertilisation requirements must be met. Failure to meet any one requirement will mean that introgression cannot occur.

371. In general terms, the hazard to the environment that might result from the movement of the genes introduced into the GM Roundup Ready® canola line GT73 to other organisms is the production of herbicide-tolerant weeds, some of which couldprove difficult to control and/or have the potential to compete with native flora thereby reducing biodiversity.

372. The potential hazards are addressed in the following sections, with respect 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

373. This section will focus on the likelihood of gene transfer (both hybridisation and introgression) from the Roundup Ready® canola to other canola crops and presents conclusions about the consequences of these risks for the environment.

Section 1.1 Nature of the gene transfer hazard

374. Transfer of the introduced genes from Roundup Ready® canola to other canola plants would present the same hazards and have the same potential environmental impacts as the presence of the genes in the Roundup Ready® canola.

375. 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.

376. The assessment of gene transfer to other canola plants has focussed on the herbicide tolerance trait. The likelihood of a hazard arising due to transfer of the regulatory sequences controlling expression of the *CP4 EPSPS* and *goxv247* genes to these species is considered to be the same as for Roundup Ready® canola, and is remote. The 3' regulatory sequences are derived from the E9 Rubisco gene of the pea plant. Although the promoter is derived from a plant pathogen (Figwort mosaic virus), it only represent a very small proportion of the pathogen genome and is not, in itself, infectious or pathogenic.

Section 1.2 Likelihood of the gene transfer hazard occurring

Outcrossing within canola

377. As there are no sexual barriers to outcrossing, cross-pollination between non-GM herbicide susceptible or herbicide tolerant (GM or non-GM) canola crops is inevitable given sufficient proximity and exposure.

378. The genetic modifications in Roundup Ready® canola are not expected to affect the rate of outcrossing to other plants compared to non-GM canola. Floral development, pollen production, pollen viability (information provided by Monsanto) and insect activity on flowers (Z. Huang, Michigan State University USA, pers. comm.). of Roundup Ready® canola are normal and do not differ from non-GM canola. Therefore the results of studies on outcrossing rates between conventional canola apply equally to genetically modified glyphosate-tolerant canola. Many studies on pollen flow use herbicide tolerance genes as markers. Hybrids resulting from outcrossing events are identified by the presence of herbicide tolerance in non-herbicide tolerant crops, or multiple herbicide tolerant types in single herbicide tolerant crops (Salisbury 2002b).

379. 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. For further details on pollination of canola refer to the "Biology and Ecology of Canola (*Brassica napus*)", available at the OGTR website.

380.

381. Recent studies have provided further support for the limited role of wind-mediated pollination in canola and the importance of insect pollinators (Cresswell et al. 2003; Hayter & Cresswell 2003; Ramsay et al. 2003) and that the architecture of the canola flower severely restricts its potential for cross-pollination by wind (Cresswell et al. 2003).

382. In Australia, honeybees (*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 (2002)).

383. However, the greatest chance of pollination by bees is from consecutive visits and that the bulk of pollen collected from any one flower is deposited on the next few flowers visited (Williams 2001).

384. Recent work in Australia supports the view that bee-mediated pollen transfer between fields is limited (Baker 2003b, J. Baker pers. comm.). Tracking of marked bees in adjacent canola fields (separated by a roadway) indicated that they had a high degree of fidelity to one field, and the majority were found in the area where they were originally marked. However, 6% of bees moved between the two adjacent fields over a 24-hour period.

385. 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 honeybees, 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, Philipps - Universitat Marburg Germany, pers. comm). There is no difference in the amount of pollen transferred by each bee species (Cresswell et al. 1995).

386. In the broad acre field situation, cross pollination between Roundup Ready® canola 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 may also occur where volunteer plants emerge after canola crops are harvested and develop to flowering stage, or where feral canola populations, resulting from seed being carried off-farm, establish along roadsides adjacent to cropping land where canola is planted.

387. 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 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 (Salisbury 2002b).

388. 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) and more recently a study conducted in the UK has reported outcrossing at 26 km (Ramsay et al. 2003). 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 2002c). Ramsay et al (Ramsay et al. 2003) have indicated that data obtained from male sterile bait plants overestimates the gene transfer into male fertile plants by about one order of magnitude.

Outcrossing rates in the Northern Hemisphere

389. Overseas studies have shown that the frequency of outcrossing varies with distance, but in general, outcrossing rates are significantly less than 1 % at 50 m from the source field and beyond (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 an indicator of pollination and subsequent seed set, the level of cross-pollination will be an overestimate. In 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; Beckie et al. 2003). At

distances up to 50 m, outcrossing rates below 0.4 % have been measured (eg Beckie et al. 2001; Champolivier et al. 1999; Downey 1999a; Downey 1999b; 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). The recent publication by Beckie et al (2003) includes a review of outcrossing rates determined in commercial field situations.

390. 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 (2003), 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.

391. Norris and Sweet (2003) conducted field plot experiments in the UK on the rate of outcrossing from glyphosate tolerant GM canola and glufosinate ammonium tolerant GM canola to an adjacent commercial field of non-GM canola. The non-GM canola was a varietal association 'Gemini'. It should be noted that varietal associations are comprised of a mixture of male sterile hybrids (~80%) and male fertile cultivars for supplying pollen for fertilisation (Ingram 2000; Ramsbottom & Kightley 1999) and are therefore, like male sterile bait plants, 'sensitive' to cross-pollination (Ingram 2000). Small plots of each GM canola (approximately 0.6 ha) were flanked by the commercial field on two sides (approximately 50ha). The GM canola plots were also surrounded by a buffer of 10 or 15m of non-GM canola. The separation between non-GM buffer was 65m on one side and 90m on the other side. Outcrossing was measured by sampling seeds (2000 per $1m^2$ quadrat) from the non-GM crop at 5m intervals, germinating these seeds and challenging with either glyphosate or glufosinate ammonium herbicide. Outcrossing rates were highest at the exterior edge of the non-GM crop and declined further from the edge. The highest outcrossing rate for the glyphosate tolerance trait from 1.33% at the crop edge (105m from the GM glyphosate tolerant canola) while for the glufosinate ammonium trait the highest rate was 0.55% (75m from the GM glufosinate ammonium tolerant canola). The total hybridisation rate was 0.32% for glufosinate ammonium and 0.99% for glyphosate and this difference was statistically significant ($\chi^2_{(1)}>300$, $P<0.01$).

392. The authors suggested a number of factors that may have contributed to the apparent difference in the outcrossing rates for the two herbicide tolerant types. They noted that the glufosinate tolerant canola used was also F_1 hybrid canola from the *barstar/barnase* system. Because the herbicide tolerance trait is only hemizygous in the male sterile parent, only a proportion of the pollen (5/8) will confer the glufosinate ammonium tolerance. Therefore scoring herbicide tolerant progeny gives an underestimate of the outcrossing rate. The authors also suggested that there might be varietal differences between the two GM crops that affected the success of cross-pollination (Norris & Sweet 2003). Most importantly however, because of the use of a varietal association, which are comprised predominantly of male sterile lines, these results represent an overestimate of outcrossing potential. Varietal associations are not used in canola cropping in Australia (P. Salisbury,

University of Melbourne, pers. comm.). A recent study by Ramsay et al (2003) in the UK employed male sterile canola to generate large data sets of maximum landscape scale gene transfer between canola. They also examined outcrossing between male fertile canola using a marker gene to track gene transfer. Their results indicate that the rate of outcrossing drops rapidly over the first few tens of metres from the edge of a field, but beyond that the decline with distance is light over longer distances and that the exact shape of the decline varied between seasons. These results are consistent with previous studies.

393. Experiments with insect exclusion cages provided strong evidence that outcrossing between canola plants, even at short distances, is mediated by insects, especially honey bees. Patterns of pollination were relatively insensitive to airborne pollen deposition. They also obtained evidence suggesting that bee to bee contact in the hive may be a major means of pollen dispersal through the foraging area of a colony. Low levels of cross pollination at 5 km and 26 km using male sterile bait plants (Ramsay et al. 2003) and the authors considered that these events were very unlikely to be mediated by bees, even when considering the maximum foraging distance and concluded that the most likely vector was the pollen beetle *Meligethes aeneus*.

394. Ramsay et al (2003)also reported differences in outcrossing to large fields or small plots (0.01 or 0.09 ha) with average outcrossing rates between large fields being <0.1 % even at relatively short distances but of the order of 1% for the small plots. For small plots the level of progeny from various outcrossing events will be proportional to the various canola sources within pollination range.

395. While most of the outcrossing studies described here used glufosinate ammonium tolerant GM canola, there is no reason to believe that in the absence of selective pressure (ie. herbicide application) the herbicide tolerance trait *per se* will affect the rate of gene transfer. Therefore the data provide a useful comparative reference for predicting the behaviour of Roundup Ready® canola.

Outcrossing rates in Australia

396. In 2000, an Australian study determined outcrossing rates between commercial fields of non-GM canola (Clearfield®) 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. This study is one of the most extensive studies of gene transfer at the commercial production scale undertaken anywhere in the world.

397. 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. The seedlings were screened by spraying with imidazolinone herbicide to determine whether pollen mediated gene transfer from source to sink fields had occurred (ie any hybrid plants that had incorporated the herbicide tolerance gene would survive the herbicide challenge).

398. 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 was 0.07%. The results indicate that gene transfer via pollen movement occurs between canola fields. However, even adjacent commercial canola fields in Australia will have much less than 1% gene transfer (Rieger et al. 2002). These results are not inconsistent with the recent results from Ramsay et al (2003)in the UK.

399. Recent results from modelling by Baker and Preston (2003) also predict that the level of gene transfer between canola fields will be low. The modelling predicted a mean frequency of resistance in a canola field adjacent to a single GM canola field (0.008-0.013%) which was consistent with the empirical data of Rieger et al. (2002) (0.009%). They also modelled the situation of a single non-GM canola field surrounded by four herbicide tolerant canola fields and predicted that the maximum level of gene transfer across the central field would be 0.13% (Baker & Preston 2003).

400. 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).

401. Recent DNA fingerprinting studies of stands of roadside canola in South Australia indicate that larger populations (ranging from 7 –25 plants) are comprised of more than one canola genotype (2 to 5). Fingerprints of maternal (leaf) and progeny (seed) material from individual plants from such stands were identical in all cases indicating a high level of self-fertilisation (Baker 2003a, J. Baker pers. comm.). These results support the conclusion that the diversity in stands was most probably as a result of multiple spills of seed rather than from outcrossing.

1.2.2 Transfer of genes between Roundup Ready® canola and conventional canola

402. Gene transfer of Roundup Ready® canola to conventional canola will result in progeny that display tolerance to glyphosate, but in all other respects the behaviour of the progeny will be determined by the genetic background of the parental varieties. There is no reason to predict that the genetic modifications introduced to Roundup Ready canola would cause it be more intrinsically invasive or persistent than conventional canola (refer to Appendix 4 for details).

403. The glyphosate tolerance trait is homozygous in Roundup Ready® canola. Outcrossing will result in hemizygous F_1 progeny. Although recent results from Halfhill et al. (2003) indicate that gene dosage can affect the level of transgene expression in canola hybrids, a range of studies have demonstrated that a single herbicide tolerance allele through outcrossing (ie hemizygous) is sufficient to provide tolerance to standard application rates of glyphosate (eg Hall et al. 2000; Norris & Sweet 2003).

404. If crossing of Roundup Ready® canola (which is homozygous for both the CP4 EPSPS and *goxv247* genes) and conventional canola did occur, 100 % of the progeny (from that cross) would be hemizygous for glyphosate tolerance genes. Backcrossing of hemizygous progeny with non- Roundup Ready® canola over subsequent generations would, in the absence of selective pressure (glyphosate application), be expected to lead to a decrease in the presence of transgenes in the population. However, given that plants resulting from a cross are self-fertile, a proportion of flowers will be self-pollinated resulting in plants homozygous for the glyphosate tolerance trait. Progeny resulting from self-fertilisation of hemizygous hybrids would result in 75% glyphosate tolerant (50% hemizygous, 25% homozygous) and 25% susceptible individuals.

405. These proportions provide an indication of the proportion of herbicide tolerant progeny that would be expected from random mating events. However, it should be noted that the likelihood of genes being transferred from Roundup Ready® canola and spreading in a

population will be influenced by a number of factors, including the level of self-pollination, physical proximity and flowering synchrony, and the vast majority of seeds from hybrid plants would be harvested along with the rest of the crop.

406. The retention of the trait in a population will also be affected by whether it provides any selective advantage. The *CP4 EPSPS* and *goxv247* genes do not provide any competitive advantage in the absence of glyphosate.

Outcrossing with other herbicide tolerant canola in the Northern Hemisphere

407. 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 widespread introduction of herbicide tolerant varieties in Canada has meant that control of volunteer canola is more complex (Derksen et al. 1999), and highlighted the pollen flow between crops that has always been present in the cropping system (Martens 2001).

408. As noted in Appendix 4, the education of farmers in Canada was generally considered to be inadequate with regard to the introduction of herbicide tolerant varieties, and growers were surprised when volunteers in paddocks neighbouring herbicide tolerant canola showed resistance to that herbicide (Simard et al. 2002), even though they could be readily controlled by the application of alternative herbicides (Beckie et al. 2001). Similarly, a number of authors have suggested that there was a lack of adequate communication to growers of guidelines for distances needed between different herbicide tolerant varieties with respect to the occurrence of unintended herbicide tolerant volunteers as a result of gene transfer by outcrossing (Simard et al. 2002; Entz & Martens 2003b; Entz & Martens 2003a; Beckie et al. 2001).

409. The fact that gene transfer has resulted in the occurrence of glyphosate volunteers where Roundup Ready® canola had not been sown has drawn attention from the popular press (eg Raine 2001; Stevenson 2002). It should be noted that the number of glyphosate tolerant canola volunteers appearing in neighbouring fields as a result of gene transfer will be minimal compared to those occurring in the field following the harvest of the Roundup Ready® canola crop.

410. 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; Beckie et al. 2003). Five herbicide tolerant types of canola have been commercialised in Canada – glyphosate (GM), glufosinate ammonium (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; Beckie et al. 2003).

411. Beckie et al (2003; 2001) conducted a study of gene transfer between adjacent (ie sharing a common border) commercial fields of Roundup Ready® canola and glufosinate ammonium tolerant canola at 11 sites in Canada grown in 1999. They sampled seed (progeny) from plants in each field and screened for outcrossing by double herbicide challenge with glyphosate and glufosinate ammonium and confirmed double tolerant phenotypes by testing for the presence of the CP4-EPSPS and phosphinothricn acetyl transferase (PAT, confers tolerance to glufosinate ammonium) using commercially available strip tests based on antibody detection. Samples were taken at distances of 0 (to 2m), 50, 100, 200, 400, 600 or 800 m along a transect from the common border. Double

resistant progeny were detected up to 400 m with outcrossing rates ranging from 1.4% at the common border to 0.04% at 400m. Outcrossing rates were markedly reduced at 50 m (between 0.15% and 0.22%). The estimated frequencies of outcrossing were generally similar between glyphosate and glufosinate tolerant fields.

412. Similar levels of gene transfer 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).

413. 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 (Downey & Beckie 2002; Friesen et al. 2003). Beckie et al (2003; 2001) also tested the seedlots used to sow the fields for the adventitious presence of double resistant seeds in the glufosinate ammonium tolerant and glyphosate tolerant seedlots. Adventitious presence of seeds tolerant to both glyphosate and glufosinate ammonium was detected in three glyphosate tolerant seedlots used at two sites at frequencies of 0.1%, 0.2% and 0.3% respectively. The threshhold for offtypes in certified seedlots in Canada is 0.25%. Further detail is provided below in the section on "Seed Production".

414. In the year following harvest (2000) three fields were screened for double resistant volunteers by sequential spraying with glyphosate and glufosinate ammonium. Double resistant volunteers were detected up to 800 m from the common border (the study limit).

415. Although seed and crop residue were cleaned from the combines between fields, some movement of harvested seed between the two types of herbicide tolerant fields could not be discounted (Beckie et al. 2003). Dietz-Pfeilstetter and Zwerger (2003) reported that even after extensive cleaning of a harvesting combine between harvesting Liberty Link (glufosinate ammonium tolerant) and Roundup Ready canola experimental plots that about 16% of the sample taken immediately after changing from the Liberty Link to the Roundup Ready plot was Liberty Link.

416. The adventitious presence of double tolerant individuals in the seedlots contributed to the incidence observed in the field. The *pat* gene from *Strepromyces viridichromogenes* which encodes PAT was detected in all double tolerant seeds. The glufosinate ammonium tolerant crops planted in this study all contain the *bar* gene from *Streptomyces hygroscopicus* which also encodes PAT. This enabled discrimination of whether glufosinate ammonium tolerant individuals arose as a result of outcrossing between the fields in 1999 or as a result of adventitious presence by molecular detection of either the *pat* or *bar* gene by polymerase chain reaction (PCR).

417. Determination of the number of volunteers pre- and post-spray enabled calculation of the frequency of double tolerant individuals. The frequency varied markedly between and within the three study sites with 'hot spots' apparent, with the highest frequencies ranging from 2.5 - >10%. For two sites the magnitude of gene transfer decreased with distance from the common borders, but this was only apparent at the farthest distance interval (400 – 600 m) for the third site.

418. The authors noted that the results of the 1999 and 2000 studies were generally not in close agreement and that the plant and seed populations sampled in 1999 represented only a small sample size (although comparable to those of many previous gene transfer studies) while those of the 2000 'whole field' volunteer screening were more robust. They also noted that the variability observed in gene transfer at the three sites measured by volunteer incidence make accurate prediction via modelling of gene transfer in canola at the

commercial scale very difficult, and that such variability should be expected because of the numerous variables that can influence gene transfer (2003). Despite the well documented occurrence of outcrossing in Canada, there has been no suggestion that this has constituted any risks to human health and safety or the environment. It should also be noted that there are no marketing requirements for segregation of GM and non-GM canola in Canada.

419. 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.

Other herbicide tolerant canola in Australia

420. There are two conventionally bred herbicide-tolerant canola varieties currently being grown throughout Australia – triazine tolerant and imidazolinone-tolerant.

Table 1: 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).

421. A significant proportion of the canola crop in Australia is triazine tolerant, with estimates of between 55% (Norton 2003b) and 70% (Table 1). Triazine tolerant canola represents up to 95% of canola production in Western Australia (Table 1, Norton 2003b).

422. 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).

423. Imidazolinone tolerant (Clearfield®, 'IT' or 'IMI') canola is resistant to imidazolinone herbicides (Group B). Clearfield® was introduced into Australia in 2000 and represents between 5 and 10% of production (Table 1, Norton 2003b). 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 differing levels of imidazolinone tolerance depending on the gene(s) present and their copy number (homozygous or hemizygous).

424. In July 2003 the Regulator issued a licence authorising the commercial release of GM glufosinate ammonium tolerant InVigor® canola in Australia (OGTR 2003). No commercial plantings of InVigor® canola have occurred in Australia to date (ie the Winter 2003 season).

Consequences of gene transfer from Roundup Ready® canola to other canola, including stacking of multiple herbicide resistance

425. Gene transfer from Roundup Ready® canola to other canola crops will result in the presence of glyphosate tolerant volunteers in fields where Roundup Ready® canola has not been grown.

426. The main consequence of gene transfer from Roundup Ready® canola to other canola crops, including other GM or non-GM herbicide tolerant crops, is that any resultant volunteers would not be controlled by glyphosate.

427. Glyphosate is registered as a range of products for use in broadacre agriculture in Australia and is widely used for non-selective weed control, including control of canola volunteers (Dignam 2001; Neve et al. 2003b, S. Powles pers. comm.).

428. Glyphosate is the only group M herbicide registered in Australia, therefore the transfer of the herbicide tolerance trait to other canola would not impact the efficacy of any other herbicide.

429. In a commercial situation low levels of outcrossing between canola varieties is inevitable.

430. If Roundup Ready® canola is grown in close proximity to other canola crops there is a high likelihood of some outcrossing resulting in glyphosate tolerant volunteers in adjacent fields where Roundup Ready® canola has not been grown. However the overall frequency of hybridisation will be low and the number of resultant glyphosate tolerant volunteers would be by the vast majority of hybrid seeds being harvested along with the crop. Such volunteers would pose the same negligible risk to human health and safety and the environment as Roundup Ready® canola (see Appendices 2, 3, 4).

431. In situations where volunteer control following a Roundup Ready® canola crop is poor, and volunteers are allowed to reach the flowering stage they will represent a secondary source for gene transfer to other canola crops (Legere et al. 2001).

432. The addition of Roundup Ready® canola to the Australian cropping system would therefore make the management of canola volunteers more complex, and it would have implications for the choice of herbicide(s) selected for control operations, not only for growers of Roundup Ready® canola, but also for growers of other canola varieties.

433. Glyphosate tolerant canola volunteers can be readily controlled by the all other herbicide and non-chemical management practices currently used to control conventional and herbicide tolerant (non-GM or GM) canola volunteers. The range of alternative herbicides to glyphosate available for control of volunteer canola are detailed in Appendix 4.

434. The control of canola volunteers in subsequent seasons is part of normal weed control operations and forms an integral part of agricultural production. The control of glyphosate tolerant canola volunteers that occur as a result of gene transfer from Roundup Ready® canola crops represents an agricultural production issue with potential economic impact in terms of alternative weed management choices, but will pose no greater risks of adverse impacts to human health and safety or the environment than conventional canola.

435. Hybridisation between Roundup Ready® canola and InVigor® canola or conventional herbicide tolerant varieties would result in accumulation or 'stacking' of genes for tolerance to multiple herbicides within the same plant.

436. Senior et al. (2002) found that stacking together glyphosate and glufosinate ammonium 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. Glufosinate ammonium is the only Group N herbicide registered in Australia, and it is only registered for use (as Liberty®) on InVigor® canola crops, not for other weed control in the broad acre setting (APVMA 2003b). Therefore progeny resulting from outcrossing between Roundup Ready® canola and InVigor® canola would be equivalent to Roundup Ready® canola with respect to volunteer control because glufosinate ammonium would not be used for volunteer control.

437. Stacking of the glyphosate tolerance trait of Roundup Ready® canola with TT or Clearfield® canola would have implications for choices of weed management strategies where triazine or ALS inhibitor herbicides are used for weed control after canola cropping. However, in situations where TT or Clearfield® canola have been grown the corresponding class of herbicide would not be effective in controlling Clearfield® or TT canola volunteers. Because the triazine tolerance trait in TT canola is maternally inherited, stacking of the glyphosate tolerance trait will only occur in the direction of Roundup Ready® canola to TT canola, and not *vice versa*.

438. Hybridisation between the existing conventional herbicide-tolerant canola varieties, InVigor® canola, and Roundup Ready® canola would result in accumulation or 'stacking' of genes for tolerance to up to four different herbicide groups within the same plant. However development of canola plants with all four herbicide tolerance traits would only be expected to occur at an extremely low frequency because it would require at least three separate hybridisation events (two events combining individual traits and a third event combining those).

439. The likelihood of multiple herbicide tolerant canola having adverse impacts on natural, undisturbed habitats is low. The presence of the glyphosate tolerance trait in canola volunteers in undisturbed habitats may have implications for the choice of herbicides in situations where glyphosate is the usual herbicide used for weed control (see Appendix 4 for further details). However, as previously stated, canola is not considered a weed of undisturbed habitats and plants with multiple herbicide tolerance will be no more weedy or invasive than single herbicide tolerant or non-herbicide tolerant canola types.

Management of gene transfer from Roundup Ready® canola, including gene stacking

440. Management of the impacts of gene transfer from Roundup Ready® canola to other canola, including the possibility of developing multiple herbicide tolerant canola by 'gene stacking', 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: attention to the control of volunteers; informed selection and rotation of herbicides

and crops; maintenance of hygiene in seeding, harvesting and transport operations; and implementation of good agronomic practices.

441. In Canada, five herbicide tolerant canola types have been commercialised, including Roundup Ready® 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). (2,4-D is currently registered in Australia, but the APVMA is conducting a review of its registration (APVMA 2003c)). Phenoxy herbicides have to be used post-emergence in cereals where volunteers contain the gene(s) for imidazolinone tolerance (Clearfield®, 'IMI') 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).

442. As detailed in Appendix 4, glyphosate can be used in combination with some herbicides, eg by tank mixing, which gives the flexibility to apply a herbicide treatment in a situation where there is a mixed weed spectrum (glyphosate tolerant and susceptible) or in situations where enhanced knockdown of difficult to control weeds is required (Howey 2002; Davies 2002; Goldwasser et al. 2003; Cumming 2002). The use of tank mixes would provide a management tool for the control of glyphosate tolerant volunteers that occur as a result of outcrossing.

443. It should also be noted that glyphosate, although a broad spectrum herbicide, would not be the herbicide of choice for the control of all weeds, as its effectiveness may be limited by environmental factors, the weed species and the developmental stage of the weeds (Adkins et al. 1998; Brown et al. 2003a; Ellis & Griffin 2003). Glyphosate is generally considered to be most effective against grasses but may be less effective against some established broadleaf species (DPIWE Tasmania 2002; eg Shaw & Arnold 2002).

444. It should also be noted that there are means by which Roundup Ready canola seed might enter a non-Roundup Ready paddock or harvest other than gene transfer through outcrossing. The first, as noted previously, is through machinery, especially seeding or harvesting equipment (Dietz-Pfeilstetter & Zwerger 2003). The second is from volunteers from a previous canola crop. A review of canola seedbanks in the UK indicated that volunteers from a previous crop could contribute as much as 1% of the yield in the current year if volunteer numbers were not rigorously controlled (Squire et al. 2003). Pekrun et al (2003) also note that volunteers from previous crops may have implications for market threshholds for adventitious presence, but that this can be readily managed by a range of practices.

445. Attention to volunteer management, proper crop rotation and herbicide management practices should limit the possibility of hybridisation between herbicide tolerant canola crops, and hence the development of multiple herbicide tolerant canola in Australia (Rieger et al. 2001; Downey 1999a; Salisbury 2002b).

Monsanto's Stewardship documents

446. As noted in Appendix 4 and Section 1.2.2 above, inadequate education of farmers may have exacerbated the issues associated with the control of unexpected herbicide tolerant volunteer canola arising from gene transfer from herbicide tolerant canola varieties in Canada, including Roundup Ready® canola.

447. Monsanto has developed a package of documents to support the management of Roundup Ready Canola: *Roundup Ready® Canola Crop Management Plan* (RRCMP), *Roundup Ready® Canola Technical Manual* (RRTM) and *Roundup Ready® Canola Resistance Management Plan* (RRRMP).

448. Draft versions of these documents have been declared as Confidential Commercial Information (CCI) under section 185 of the Act. The company has subsequently developed the documentation further but they cannot be finalised until regulatory approvals are received from the Regulator and the APVMA. However Monsanto have indicated that the final versions of these documents will be made publicly available as soon as possible, if and when the release of Roundup Ready® canola is approved by the Regulator and 'Roundup Ready herbicide by Monsanto' is registered by the APVMA, and the relevant regulatory requirements have been incorporated.

449. To minimise gene transfer between adjacent non-GM and GM canola crops, Monsanto recommends that a band of at least 5m of the GM crop be slashed and cultivated prior to flowering or that at least 5m of the non-GM crop be harvested and processed as part of the GM crop. Monsanto also highlights the need to monitor and control volunteers in paddocks adjacent to where Roundup Ready® canola has been grown, in areas where seed spillage has occurred, where machinery may have deposited viable seed, and in areas where grazing animals excrete for up to 7 days after digesting canola seed.

450. These plans recommend that farmers pay particular attention to volunteer management and herbicide selection, and to avoid the development of multiple herbicide tolerance in order to effectively control canola volunteers. To minimise the potential for gene transfer they recommend that growers:

- treat the area immediately adjacent to the Roundup Ready canola as GM for subsequent volunteer control;
- communicate with neighbours if a GM crop is grown along a boundary adjacent to a neighbouring canola crop;
- clean machinery and trucks to reduce spread of GM seed;
- assess fields and adjacent areas for the presence of volunteers and choose appropriate management techniques (eg herbicides, grazing or cultivation) to remove volunteers prior to flowering;
- use crop rotations that allow removal of volunteers; and
- keep accurate field records.

451. The recommendations in the RRCMP and RRTM for the implementation of a 5m 'buffer' or gap between Roundup Ready® canola and other canola crops relate to addressing possible market requirements or thresholds regarding the adventitious presence of GM canola and not human health and safety or environment issues.

452. The presence of a 5m buffer between adjacent GM and non-GM canola fields would not eliminate gene transfer between the two crops. It is well established that the rate of cross-pollination between canola decreases significantly over the first 5-10 metres. The experimental data of Rieger et al. (2002) and the modelling work of Baker and Preston (2003) support the conclusion that the amount of gene transfer between commercial canola fields, in the absence of any containment measures, would be well below 1% on a paddock basis.

453. 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 if 1 m of the adjoining edge of the crop was removed after flowering.

454. Monsanto's recommendation that where a GM canola crop is grown along a boundary fence line adjacent to a neighbouring canola crop, the farmer should notify the adjoining land owner relates to arrangements between individual growers that are obviously outside the scope of this assessment.

Seed production – Canadian Experience

455. A recent Canadian study reported levels of contamination in certified seed lots of canola which exceed industry standards (Downey & Beckie 2002). Seventy seed samples from 14 supposedly non-herbicide tolerant (ie herbicide susceptible) varieties were sprayed with herbicides to screen for the presence of genetically modified herbicide tolerance genes, including glyphosate tolerance. 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.

456. Glyphosate tolerant seedlings were present in 50% of samples, 20% contained glufosinate ammonium tolerant seedlings and 15% had seedlings that were tolerant to both herbicides.

457. 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 analysed. 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 seedlot samples, only one 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.

458. The study by Beckie et al (2003) examining outcrossing between commercial fields in Canada also detected the adventitious presence of double tolerant glyphosate and glufosinate ammonium tolerant seeds in three certified glyphosate tolerant seed lots. However they did not detect any adventitious presence of double tolerant seeds in two seed glufosinate ammonium tolerant hybrid seed lots. They suggested that the greater minimum isolation distance of 800 m required for the production of certified seed of hybrid canola in Canada (CSGA 2003), as opposed to the 100 m minimum isolation required for pedigree-derived cultivars, may have reduced the presence of off-types in these seed lots. Minimum isolation requirements for certified canola seed in Australia are based on OECD Rules and

Directives and are 100 m for open pollinated pedigreed seed and 300 m for hybrid seed (Glover 2002).

459. 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.

460. Although instances of significant seedlot contamination in Canada were attributable to causes other than gene transfer, the evidence from Canada indicates that outcrossing from Roundup Ready® canola crops to certified seed production plots may result in low levels of adventitious presence of glyphosate tolerant canola 'off types' in certified canola seed lots. The frequency of such outcrossing will be affected by the isolation measures adopted by seed producers and the proximity of Roundup Ready® canola crops. These results may also have implications, in Australia and elsewhere, for the minimum isolation requirements employed in the production of certified canola seed (both GM and non-GM) to ensure that desired purity standards are achieved.

461. The presence of such Roundup Ready® canola off types would result in glyphosate tolerant canola volunteers after harvest of the non-Roundup Ready® crop. The implication of this would be that these volunteers would not be controlled by glyphosate. As for the control of glyphosate tolerant canola volunteers that occur as a result of gene transfer from Roundup Ready® canola crops, the control of Roundup Ready® canola volunteers as a result of adventitious presence in seed lots represents an agricultural production issue with potential economic impact in terms of alternative weed management choices. The presence of Roundup Ready® canola off types in non-Roundup Ready canola seed lots would pose the same negligible risk to human health and safety and the environment as Roundup Ready® canola (see Appendices 2, 3, 4).

Seed production in Australia

462. Until recently the Commonwealth Government delegated the operational functions of the OECD Seed Certification Scheme to a number of organisations, mainly State and Territory departments. In February 2003 the operational aspects of the OECD Seed Certification Scheme in Australia were delegated to the industry owned Australian Seeds Authority (ASA 2003).

463. As noted above, isolation requirements for certified seed production are based on OECD Seed Certification Scheme - Rules and Directives (OECD 2003), however individual proprietary users (seed producers) will implement measures based on their own quality assurance and control needs. Industry standards for isolation and quality assurance relating to production and marketing of seed for sowing will reduce the likelihood of outcrossing resulting in glyphosate tolerant 'off types' in non-Roundup Ready® canola seed lots.

464. The Seed Industry Association of Australia (SIAA) has a national code of practice for labelling of seed for sowing and marketing (SIAA 1999) and is currently developing an industry standard for the adventitious presence of genetically modified seed in seed lots. This standard has not yet been finalised but will be determined on the basis of meeting domestic and international seed and bulk commodity market requirements.

465. Monsanto has indicated that the production of Roundup Ready® canola seed for distribution to growers will be conducted according to the industry standards for the production of certified canola seed, and that strict quality assurance protocols will be

followed. Roundup Ready® canola seed production plots for breeders' seed will be isolated from other canola crops by a minimum distance of 400 m. These measures will minimise the level of contamination of Roundup Ready® canola seed by surrounding canola crops and also limit the potential for gene transfer to occur from Roundup Ready® canola seed production plots to surrounding canola crops.

Section 1.3 Conclusions regarding gene transfer to other canola plants

466. 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 (up to 2.6 km under Australian conditions), but at extremely low levels.

467. In a commercial situation, low levels of outcrossing between canola varieties is inevitable. However the transfer of the CP4 EPSPS and *goxv247* herbicide tolerance genes from Roundup Ready®canola to other canola will not confer a competitive or ecological advantage to these plants in the absence of glyphosate, and the hazards are the same as for Roundup Ready® canola.

468. Gene transfer from Roundup Ready® canola to conventional seed production plots may result in very low levels of adventitious presence of glyphosate tolerant canola seeds as 'off types' in non- Roundup Ready® canola seed lots. Industry standards for isolation and quality assurance relating to production and marketing of seed for sowing will reduce the likelihood of outcrossing resulting in glyphosate tolerant 'off types' in non-Roundup Ready® canola seed lots.

469. If gene transfer from Roundup Ready® canola to conventional canola (either commercial or seed crops) did occur as a result of outcrossing, the hazards will be the same as those for Roundup Ready® canola;

470. The control of canola volunteers in subsequent seasons is part of normal weed control operations and conventional agricultural practice. Glyphosate tolerant canola volunteers can be readily controlled by the all other herbicide and non-chemical management practices currently used to control conventional and herbicide tolerant (non-GM or GM) canola volunteers.

471. 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. Stacking of the glyphosate tolerance trait with the other herbicide tolerance traits in canola would have implications for the choice of herbicide(s) used for subsequent weed control operations on-farm.

472. The development of herbicide tolerant volunteers can be minimised by good management practices both on and off farm.

473. Roundup Ready® canola volunteers that arise as a result of gene transfer will represent an agricultural production issue with potential economic impact and increased complexity in terms of implementing alternative weed management strategies, but will pose no greater risks to human health and safety or the environment than conventional canola.

474. The risk associated with gene transfer to other canola is therefore concluded to be negligible and no management conditions to limit gene transfer are proposed for this release.

SECTION 2 TRANSFER OF INTRODUCED GENES TO OTHER PLANTS

475. This section will focus on the likelihood of gene transfer, including introgression, from Roundup Ready® canola to related Brassicaceae species and make conclusions about the consequences of any such gene transfer in terms of adverse impacts on the environment.

Section 2.1 Nature of the gene transfer hazard

476. 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 genes (CP4 EPSPS and *goxv247* genes)

477. Plants would become tolerant to glyphosate. This could have an impact in situations where glyphosate is extensively used for weed control.

Promoters and other regulatory sequences

478. If gene transfer did occur, there is a possibility of unintended or unexpected effects if the introduced regulatory sequences altered the expression of endogenous plant genes. Some regulatory sequences introduced into Roundup Ready® canola are derived from plant pathogens (Figwort mosaic virus).

Section 2.2 Likelihood of the gene transfer hazard occurring

479. For transgenes to flow from Roundup Ready® canola to other plants and persist in the recipient plants, the first step is the production of interspecific hybrids. The proportions of herbicide tolerant and susceptible progeny expected to be produced from crosses with Roundup Ready® canola and related brassicaceous species are the same as for a cross with *B. napus* (refer to Section 1 of this Appendix). The CP4 EPSPS and *goxv247* genes are completely linked and would be passed from one plant to another as a single locus.

480. Following the initial hybridisation event, efficient gene transfer 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 depend on the fertility, genomic structure, vigour of the progeny, sexual compatibility of progeny with the wild type and the transmission of Roundup Ready® canola genes within successive generations.

481. 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. Nevertheless, repeated backcrossing of the hybrid with wild plants can lead to gradual introgression of the gene in question into the wild population.

482. The most likely possibility of gene transfer to other plant species would be transfer to other *Brassica* species or sexually compatible Brassicaceae species (at a lower level), although both are 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.

483. Table 2 summarises the potential for gene transfer between canola (*B. napus*) and Brassicaceae species found in Australia (Salisbury 2002b)

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

484. Gene transfer is possible from *B. napus* canola to *B. napus* forage rape and vegetables such as swedes, rutabaga and Siberian kale (Salisbury 2002b). However, as *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

485. 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 transfer between canola (B. napus) & Australian Brassicaceae species (Salisbury 2002)

Category	I	II	III	IV	V	VI
Tribe	*Brassiceae*	*Brassiceae*	*Brassiceae*	*Brassiceae*	*Brassiceae*	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	*Brassica rapa* *Brassica juncea*[1]	*Raphanus raphanistrum* *Hirschfeldia incana* *Sinapis arvensis*	*Brassica fruticulosa* *Brassica nigra* *Brassica tournefortii* *Diplotaxis muralis* *Diplotaxis tenuifolia* *Rapistrum rugosum*	*Brassica oxyrrhina* *Diplotaxis tenuisiliqua*	*Conringia orientalis* *Carrichtera annua* *Cakile maritima*	*Capsella bursapastoris* *Cardaria draba* *Lepidium sp.* *Myagrum perfoliatum* *Sisymbrium orientale* *Sisymbrium irio* *Sisymbrium erysimoides* *Sisymbrium officinale*
Condiment, fodder & vegetable species	Forage *B. napus*[1] *B. napus* vegetables[1] *B. rapa* vegetables[1] Condiment *B. juncea*[1]		*Brassica alboglabra*[3] *Brassica chinensis*[4] *Brassica nigra* *Brassica oleracea* *Brassica pekinensis*[4] *Raphanus sativus* *Sinapis alba*			

DECREASING SEXUAL COMPATIBILITY →

[1] considered likely to happen over a period of time if the species are in physical proximity and have flowering synchrony.

Frequency of interspecific hybrids approx. 10^{-4} to 10^{-8}. Likelihood of subsequent introgression or formation of fertile amphidiploids significantly less again.

This species is sometimes considered to be a subspecies of *B. oleracea*.

These species have sometimes been considered to be subspecies of *B. rapa*.

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

Category	I	II	III	IV	V	VI
Tribe	*Brassiceae*	*Brassiceae*	*Brassiceae*	*Brassiceae*	*Brassiceae*	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*				
Native species						*Arabidella (6 sp.)* *Balbaretinia (1 sp.)* *Barbarea (2 sp.)* *Blennodia (25 sp.)* *Cardomine (5 sp.)* *Carinavalva (1 sp.)* *Cheesemania (1 sp.)* *Cuphonotus (2 sp.)* *Geococcus (1 sp.)* *Harmsiodoxa (3 sp.)* *Irenepharsus (3sp.)* *Lepidium (35 sp.)* *Menkea (6 sp.)* *Microlepidium (2 sp.)* *Pachymitus (1 sp.)* *Phlegmatospermum (4 sp.)* *Rorippa (4 sp.)* *Scambopus (1 sp.)* *Stenopetalum (9 sp.)*

→ DECREASING SEXUAL COMPATIBILITY →

Brassica rapa

486. *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 (Groves et al. 2000; Groves et al. 2002; Holm et al. 1997; Hyde-Wyatt & Morris 1989) however other reports indicate that it is not a widespread agricultural weed (Hussey et al. 1997; Salisbury 2002b).

487. *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, Tasmanian Department of Primary Industries Water and Environment, pers. comm.). Therefore, the possibility that the genes encoding tolerance to glyphosate will be transferred to hybrids and introgress into weedy *B. rapa* populations is most likely to occur in Tasmania.

488. 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 2002b), but it is still grown as a forage crop ('forage rape') in Australia, sometimes as a mixture with *B. napus*. *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)

489. *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 2002b).

490. 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; Brown & Brown 1996; Halfhill et al. 2002; Jorgensen et al. 1996; Warwick et al. 2003). However hybrids between *B. napus* and *B. rapa* have not been reported to date in Australia, except putatively in plant breeders nurseries (Salisbury 2002b).

491. Gene transfer 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 (Salisbury 2002b; Jorgensen & Andersen 1994). 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).

492. 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*.

493. 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. Recently Wilkinson et al. (2003) screened fields of rapeseed in the UK infested with *B. rapa* for hybrids and found 46 hybrids from 2388 *B. rapa* plants (1.9% surviving hybrids 'in crop'). Investigation of hybridisation from a GM glufosinate ammonium tolerant canola line to *B. rapa* under field conditions found a hybridisation frequency of 3.3% (see OGTR 2003).

494. Intermediate rates of hybrid formation seem to be achieved when there are mixed populations of *B. rapa* and *B. napus*, either in experimental plantings or natural populations. In a 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% in experimental mixed stands of *B. napus* and *B. rapa* in Norway.

495. The highest rates of hybridisation have been observed for single *B. rapa* plants growing in fields of *B. napus* with up to 93% of F_1 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*.

496. When *B. rapa* is separated from *B. napus* the rate of hybridisation is low. Norris and Sweet (2003) 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 GM canola trial sites in Tasmania have failed to detect any gene transfer from GM canola to *B. rapa* (Rieger et al. 2002; Agronico 2002).

497. In field experiments conducted in Canada with individual *B. rapa* plants positioned within or adjacent to (0.5m from the edge) plots of Roundup Ready® *B. napus* hybridisation rates were between 3.4% - 8.3% (*B. rapa* as mother), with the lowest rates at the margin (Warwick et al. 2003). All F_1 hybrids were morphologically similar to *B. rapa* and had reduced pollen viability (average 54%). However a large proportion of the hybrids were self-fertile.

498. 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.

499. 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. F_1 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).

500. 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.

501. 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 cornfield 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.

502. 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.

503. 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.

504. 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).

505. Recent studies have provided evidence that the fitness of hybrids between *B. napus* and *B. rapa* may be strongly frequency dependent (Hauser et al. 1998b; Hauser et al. 1998a; Hauser et al. 2003; Pertl et al. 2002).

506. Pertl et al. (2002) measured the effect of planting density and different proportions of *B. napus*, *B. rapa* and their F_1 hybrids on their fitness when pollinating *B. rapa* plants. They found that flowering periods of the two species and the F_1 hybrids overlapped extensively and that plants at low density ($16m^{-2}$) produced more flowers and flowered later than at high planting density ($100m^{-2}$). F_1 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 F_1 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 F_1 hybrids in the field. Hauser et al. (2003) also demonstrated that F_1 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 F_1 hybrids producing many more seeds in mixtures than in pure stands.

507. In contrast male fitness was found to be much lower in the F_1 hybrids. Although the number of pollen grains produced per flower was similar among *B. rapa*, *B. napus* and the

F_1 hybrids, pollen viability was much lower in the F_1 hybrids than the parents and declined slightly over the season. Furthermore both F_1 hybrids and *B. napus* almost only sired offspring when at high frequencies themselves. The number of F_1 and backcross offspring was also less than expected at low planting densities.

508. The implication of these results is that although female fitness may be much higher in F_1 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 F_1 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 F_1 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.

509. 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).

510. 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. 1998b; Hauser et al. 1998a; Hauser et al. 2003; Snow et al. 1999; Hansen et al. 2001; Norris & Sweet 2003). 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 F_1 generation.

511. 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.

512. A recent study modelled the rate of outcrossing from *B. napus* to riparian (riverside) populations of *B. rapa* over the whole of the UK (Wilkinson et al. 2003). *B. rapa* tends to be found in the UK as semi-natural, wild communities along riversides in contrast to its distribution in Australia. This study was based on empirical data for the incidence of *B. napus – B. rapa* hybrids for 8 sympatric populations (ie occurring close together). For each of these sympatric sites the *B. rapa* population was within 30 m of the canola field. From these sites 47 out of 3230 *B. rapa* plants were identified as being inter-specific hybrids, or 1.4%. Using satellite imagery the authors estimated that there are 1.8 million riparian *B. rapa* plants sympatric with rapeseed fields in the UK including an estimated 26,000 *B. napus – B. rapa* hybrids.

513. Airborne pollen dispersal profiles were used to construct a mathematical model for the possible rate of hybridisation with riparian *B. rapa* populations over greater distances. Pollen density measures were converted to hybrid frequency using the empirical data from the short distance observations. However, as noted previously, other work suggests that the majority of long distance pollination of canola is mediated by insect pollinators (eg Ramsay

et al. 2003). The calculated annual hybrid formation rate in riparian *B. rapa* populations was calculated to be 0.0065%.

514. The study also extrapolated the empirical sympatric observations and the long distance modelling to arrive at an estimate for canola to *B. rapa* hybidisation in crops in the UK. A survey of fields north of the Humber River revealed *B. rapa* in 1.8% of fields and that canola is grown in 1.8% of fields. Using these proportions and the assumption that *B. rapa* is only "reliably apparent" in canola the authors calculated that 21% of canola fields contain *B. rapa*. Using these parameters their model predicts an overall 'in-crop' rate of canola-*B.rapa* hybrid formation of 1.9% per annum.

515. 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).

516. 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*. F_1 hybrids backcrossed with *B. rapa* at similar rates. Recent results of Hansen et al (2003) demonstrated that introgression can lead to incorporation of the *B. napus* C genome in the *B. rapa* genome.

517. In summary, where *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 influenced by the frequency of parents and hybrids.

Brassica juncea

518. *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 2002b). *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*.

519. *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 progeny seeds from *B. juncea* were hybrids (Jorgensen 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

520. 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 2002b). 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 genes to be acquired from canola (Salisbury 2002b). Furthermore, these plants are not recognised as weeds in agricultural environments in Australia.

521. *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 2002b).

2.2.3 Introgression of genes into other Brassicaceae species

522. Hybrids between canola and a number of Brassicaceae species have been reported following sophisticated hand pollination and embryo rescue techniques (OGTR 2002; Rieger et al. 1999; Salisbury & Wratten 1997; Scheffler & Dale 1994). However, this does not give an accurate indication of the potential for cross-pollination and introgression in the field.

523. 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 2002b; Norris & Sweet 2003). The potential for transgene introgression in these species is discussed in detail below.

Raphanus raphanistrum

524. *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 and represents a significant economic problem in canola and other crops (Blackshaw 2001). *R. raphanistrum* can exhibit considerable seed dormancy of up to 6 years (Cheam & Code 1998; Murphy 2001). It is a declared weed in many areas of NSW and its entry to Western Australia is prohibited (The National Weeds Strategy 2003). 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).

525. Hybrids between canola and *R. raphanistrum* have been reported in the field both in Australia (Rieger et al. 2001; Rieger et al. 1999) and overseas (Chevre et al. 1996; Chevre et al. 1997; Chevre et al. 1998; Chevre et al. 1999a; Chevre et al. 1999b; Chevre et al. 2000; Darmency et al. 1995; Darmency et al. 1998; Warwick et al. 2003). *R. raphanistrum* is self-incompatible and therefore open to fertilisation from other pollen sources (Sampson 1967).

526. 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)

527. When *R. raphanistrum* was grown in fields of canola in France, Chèvre et al. (1999a; 2000) reported estimated hybrid frequencies of 3 x 10^{-5} to 3 x 10^{-7} with canola as the pollen donor.

528. 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 F_1 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 F_1 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*".

529. Warwick et al (2003) also investigated 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. F_1 hybrids were identified by glyphosate tolerance.

530. In two 10m x 10m experimental field plots *R. raphanistrum* at 1 plant/m^2 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 x 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).

531. No hybrids were detected from 22,114 *R. raphanistrum* seeds collected in or near commercial glyphosate tolerant canola crops (Warwick et al. 2003).

532. Norris and 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.

533. 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 x 10^{-5} with canola as the pollen donor.

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

534. 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. 1999a; Chevre et al. 2000). 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 %.

535. 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 (Gueritaine et al. 2003a). These F_1 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* are less likely than both parents to emerge and survive to reproduce under agronomic and natural conditions (Gueritaine et al. 2003a).

536. Several studies have demonstrated that there is significant variation between cultivars of canola and *R. raphanistrum* genotypes in terms of hybridisation (Baranger et al. 1995a; Gueritaine et al. 2003b; Gueritaine & Darmency 2001). Gueritaine and Darmency (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).

537. 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.

538. 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.

539. As 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 Roundup Ready® canola and *R. raphanistrum* also have small seeds and these are not collected at harvest, glyphosate tolerant hybrid seeds could remain in the field. Any glyphosate tolerant hybrids remaining in the field following a Roundup Ready® canola crop would be just as susceptible as Roundup Ready® canola volunteers and would be readily controlled by a variety of herbicides and cultural control methods (see Appendix 4).

540. 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. Germination of hybrid seeds declined in the same manner, but was around 1 % after the first year and less than 0.1 % after 3 years.

541. 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. 1999a).

542. Gueritaine et al. (2002) recently examined the fitness of the backcross 6 (BC6) generation under field conditions. The BC5 generation was derived from an original F_1 hybrid from a cross of *R. raphanistrum* (pollen donor) x glufosinate-tolerant canola (female) and 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 (Gueritaine et al. 2002).

543. Downey (1999b; 1999a) 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. (Chevre et al. 2000) 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

544. *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 2002a). 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).

545. 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 & 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 $12m^2$). 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 et al. 1996a). 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).

546. 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. 1999a). 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 2002a).

Sinapis arvensis

547. *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).

548. 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.

549. 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. 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* (2002).

550. 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. 1999; Moyes et al. 2002). 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 %.

551. 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.

552. 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).

553. 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 transfer is likely to occur between canola and *S. arvensis* in the field (Downey 1999b; Downey 1999a).

Other weedy species in the Brassicaceae family

554. 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 2002a).

555. The possibility of gene transfer from *B. napus* to other *Brassica* or brassicaceous species, that it cannot hybridise with directly, via an intermediate or 'bridge' species has also been considered. The most likely candidates for such a route of transfer are those which can hybridise readily with *B. napus*, ie *B. rapa* or *B. juncea*.

556. Although there is a significant body of research on the potential for natural hybridisation between *B. napus* and a range of brassicaceous species, there have only been limited investigations of hybridisations between these other species, and these are the result of specific breeding programs (eg, Choudhary et al. 2002). Hybrids between *B. rapa* and *B. tournefortii* (Choudhary & Joshi 2001a), and *B. carinata* (only with B. carinata as the female parent, Choudhary et al. 2000), and between *B. juncea* and *B. tournefortii* (Choudhary & Joshi 2001b) have been reported, but only by deliberate hand pollination, and progeny from such crosses have reduced fertility. Other crosses that have been

achieved have also relied on hand pollination or other sophisticated techniques (eg Nagpal et al. 1996, P. Salisbury pers comm).

557. The likelihood of gene transfer from *B. napus* to a non-compatible brassicaceous species via an intermediate species is considered to be extremely low. Such a transfer necessitates the occurrence of successful hybridisation between *B. napus* and the 'bridge' species, survival of hybrid progeny, probably stable introgression, then requires another successful hybridisation between the interspecific hybrid or introgressed population and the third species, with the attendant prerequisites of proximity and flowering synchrony.

Possible consequences of gene transfer to sexually compatible plants

558. *B. rapa*, *B. juncea*, *R. raphanistrum*, *H. incana* and *S. arvensis* are all principally weeds of agricultural cropping or disturbed habitats in Australia. The introgression of the glyphosate tolerance genes from Roundup Ready® canola into these species will not make them more invasive or persistent, and would not provide them with an ecological advantage in the absence of selection with glyphosate.

559. 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, further mitigating against any increased weediness as a result of gene transfer from the glyphosate tolerant Roundup Ready® canola.

560. Glyphosate is widely used for weed control in agricultural, horticultural, industrial and other situations in Australia, including the control of brassicaceous weeds (glyphosate is one of the herbicides recommended for control of B. rapa in Tasmania, Anon. 2002).

561. Glyphosate tolerant hybrids would be most likely to arise either in or adjacent to paddocks in which Roundup Ready® canola is cultivated, and in these situations glyphosate would not be used for weed control post-harvest because it would not control Roundup Ready® canola volunteers. Measures taken to control Roundup Ready® canola volunteers would also eliminate any glyphosate tolerant hybrids.

562. As outlined above and in Appendix 4, glyphosate is not the herbicide of choice for the control of all weeds, and combination of glyphosate with some herbicides can be used in situations where there is a mixed weed spectrum or enhanced knockdown of difficult to control weeds is required. The use of tank mixes would provide a management tool for the control of glyphosate tolerant hybrids that may occur as a result of outcrossing.

563. If gene transfer did occur, glyphosate tolerant hybrids could be managed using the range of alternative herbicides and non-chemical management techniques currently used to control brassicaceous weeds. The range of alternative herbicides to glyphosate are detailed in Appendix 4.

564. Accordingly, gene transfer from Roundup Ready® canola resulting in glyphosate tolerant interspecific hybrids would not result in an adverse impact on the environment that cannot be managed. Although it would have implications for choice of herbicide(s) in situations where glyphosate is the principal strategy or is substantially relied on for control of *Brassica* or brassicaceous weeds. It should be noted that over reliance on one herbicide si not consistent with accepted principles of integrated weed management (see Appendix 6 for further details). Control of these weeds is predominantly an agricultural production issue and this represents an economic impact.

565. The risk of glyphosate-tolerant hybrid populations threatening undisturbed natural habitats is negligible since the *Brassica* or brassicaceous weeds capable of hybridising with *B. napus* do not tend to invade and persist in natural undisturbed habitats in Australia. Such hybrids would only have a selective advantage in situations where they are exposed to glyphosate. While the survey results of Dignam (2001) indicate that glyphosate is the most widely used herbicide in National Parks, it should be noted that broadcast spraying of vegetation does not occur in undisturbed habitats, and weeds are removed by spot spraying. In addition the survey results of (Dignam 2001)) also indicate that 93% of National Parks are not subjected to weed management operations. Therefore even if glyphosate tolerant hybrids occurred in an undisturbed habitat it is unlikely that they would be exposed to selection by glyphosate.

Section 2.3 Conclusions regarding gene transfer to other plants

Brassica napus vegetables and forage rape

566. 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 rarely flower as they are usually consumed prior to flowering or seed production.

567. Gene transfer from the glyphosate tolerant Roundup Ready® 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 proposed for this release.

Brassica rapa

568. If *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 the parental species and hybrids in a population.

569. In a commercial situation the overall likelihood of some transfer of the introduced genes from Roundup Ready® canola to the closely related *B. rapa* is high if they are in close proximity. However, the frequency of outcrossing is expected to be even lower than for conventional (non-GM) canola because of the lower incidence of *B. rapa*. 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 in that State.

570. Transfer of the glyphosate tolerance genes from Roundup Ready® canola to *B. rapa* will not result in adverse impacts to human health and safety or the environment. Resultant hybrids would not be more invasive or persistent, and would not have an ecological advantage in the absence of selection with glyphosate.

571. In attributing a risk value for gene transfer of the glyphosate tolerance genes from Roundup Ready® canola to *B. rapa*, its relative weediness, distribution and potential to persist in the environment has been taken into account. The risk associated with gene

transfer to *B. rapa* is concluded to be very low and no management conditions are proposed for this release.

B. juncea

572. The likelihood of some transfer of the introduced genes from Roundup Ready® canola to the closely related *B. juncea* in a commercial situation is also high if they are in close proximity. The rate of hybridisation and introgression will be influenced by the distribution, proximity and genetic compatibility of each species. The frequency of outcrossing is expected to be even lower than for conventional (non-GM) canola or *B. rapa* because of the lower incidence of this species and the reduced fitness of any hybrid progeny.

573. Transfer of the introduced genes from Roundup Ready® canola to *B. juncea* will not result in adverse impacts to human health and safety or the environment. Resultant hybrids would not be more invasive or persistent, and would not have an ecological advantage in the absence of selection with glyphosate. The risk associated with gene transfer is therefore concluded to be negligible and no management conditions are proposed for this release.

B. oleracea

574. 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 Roundup Ready® canola to *B. oleracea* is concluded to be negligible and no management conditions are proposed for this release.

Brassicaceous weeds

575. 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 2002a), other brassicaceous species are highly unlikely to hybridise with *B. napus*.

576. Inter-specific crosses between canola (either conventional or GM) and *R. raphanistrum* occur 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 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*.

577. Inter-specific crosses between canola (conventional or GM) and *H. incana* are 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.

578. 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.

579. If outcrossing and introgression of the introduced genes from Roundup Ready® canola 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 glyphosate herbicide.

580. The likelihood of transfer and introgression of genes from Roundup Ready® 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. Resultant hybrids would not be more invasive or persistent, and would not have an ecological advantage in the absence of selection with glyphosate. However even if glyphosate tolerant hybrids did occur they could be controlled using the range of alternative herbicides and non-chemical management techniques currently used to control brassicaceous weeds.

581. In attributing a risk value for gene transfer of the glyphosate tolerance genes from Roundup Ready canola to any of *R. raphanistrum*, *H. incana* or *S. arvensis*, their relative weediness and potential to persist in the environment has been taken into account. The risk associated with gene transfer to these species is concluded to be very low and no management conditions are proposed for this release.

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

582. 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, HGT). There are no evidence of horizontal gene transfer of intact genes between plants and mammalian cells (Thomson 2001), 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.

583. Transduction is a bacterium-virus interaction that can mediate gene transfer between bacteria in the environment (eg. 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).

584. Conjugation is a mechanism of cell-to-cell interaction that can mediate gene transfer between bacteria in the environment (eg. 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 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 (eg. transposons that function in both plants and prokaryotes, Nielsen et al. 1998).

585. 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 Roundup Ready® canola

586. Both of the genes present in Roundup Ready® canola 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.

CP4 EPSPS gene

587. The CP4 EPSPS herbicide tolerance gene was originally isolated from the common soil bacteria *Agrobacterium* sp. strain CP4. Some *Agrobacterium* spp. are pathogens of plants – *A. tumefaciens* and *A. rhizogenes* which cause crown gall and hairy root disease respectively, but are not recognised as pathogens of humans or other animals (Organisation for Economic Co-operation and Development (OECD) 1999). *Agrobacterium radiobacter* strains have been implicated as opportunistic human pathogens in immuno-compromised individuals or in the clinical setting (Alnor et al. 1994; eg Manfredi et al. 1999).

588. However the CP4 EPSPS gene is the only gene from *Agrobacterium* sp. strain CP4 introduced to Roundup Ready® canola, it only represents a very small proportion of the pathogen genome and is not, in itself, infectious or pathogenic. EPSPS genes are already present naturally in bacteria and plants. Transfer of the CP4 EPSP gene would not present a hazard to human health or the environment.

***goxv247* gene**

589. The *goxv247* herbicide tolerance gene was originally isolated from the soil bacterium *Ochromobactrum anthropi* (formerly *Achromobacter* sp. strain LBAA). *O. anthropi* is recognised as an opportunistic pathogen of humans, especially immuno-compromised patients (Teyssier et al. 2003; Alnor et al. 1994; eg Mahmood et al. 2000).

590. However the *goxv247* gene is the only gene from *O. anthropi* introduced to Roundup Ready® canola, it only represents a very small proportion of the pathogen genome and is not, in itself, infectious or pathogenic. Glyphosate oxidoreductase genes are already present naturally in soil bacteria and transfer of the *goxv247* gene would not present a hazard to human health or the environment.

Promoters and other regulatory sequences

591. If gene transfer occurred, there is a possibility that 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.

592. The regulatory sequences present in Roundup Ready® canola (see Appendix 1 for details) are derived from Figwort mosaic virus (P-CMoVb promoter) and the pea plant (transcriptional termination signals of the E9 Rubisco gene). As described in Appendix 1, the P-CMoVb promoter is thought to be functionally equivalent to the 35S promoter of Cauliflower mosaic virus (CaMV) (see Appendix 1 for details). Both of these sequences and the organisms they were derived from are frequently encountered in the environment. While the P-CMoVb sequence is derived from the plant pathogen, Figwort mosaic virus, it only represents a very small proportion of the pathogen genome and is not, in itself, infectious or pathogenic.

593. While Ho et al. (2000) have postulated that there are risks posed through recombination of viral promoters used in GM plants, such as 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 viruses such as CaMV and CMoVb and already ubiquitous in the environment (Hodgson 2000a).

Section 3.2 Likelihood of the gene transfer hazard occurring

594. Horizontal gene transfer can occur in nature between sexually incompatible organisms, however most gene transfers have been identified through phylogenetic analyses (Ochman et al. 2000; Smith et al. 1992; Worobey & Holmes 1999) and occur over evolutionary time scales of millions of years (Lawrence & Ochman 1998; Doolittle 1999). Evidence from such phylogenetic analyses of 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 instances of HGT identified have been between viruses (Lai 1992) or bacteria (Ochman et al. 2000). Less frequently, viruses have transferred genes to their hosts.

595. Theoretically, horizontal gene transfer from GM canola to other organisms, including humans and microorganisms is possible, but it is extremely unlikely.

596. This is because horizontal gene transfer does not happen frequently, as inferred from phylogenetic analyses, and because there are a number of barriers including temporal and spatial, biochemical, physiological, transfer, establishment, expression and evolutionary barriers (Nielsen 1998).

597. 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).

598. 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 (Berndt et al. 2003); 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.

599. 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

600. 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.

601. Canola oil is the only fraction of Roundup Ready® canola plants to be eaten as food by humans. Canola oil undergoes extensive processing and the oil from Roundup Ready® canola protein was below the limit of detection *ie*. less than 0.00013% of oil (Nickson et al. 1994, Monsanto Unpublished). Hence it is highly unlikely that oil contains any DNA.

602. Since humans will not be exposed to significant DNA from Roundup Ready® canola via the digestive system, the possibility of gene transfer to human cells or microoganisms in the human gut was considered highly unlikely.

603. Netherwood (2002) investigated the possibility that plant DNA could survive in the human gut and transfer genes to gut microflora. Despite exhaustive attempts to culture microbes from ileal digesta within colostomy bags, no bacteria that had taken up the transgene could be cultured (see ACRE advice September 2003). In people with intact digestive systems DNA was rapidly degraded in the colon and none could be detected in faeces. The Food Standards Agency UK concluded from this study that it was "extremely unlikely that functional DNA from GM food can be taken up by bacteria in the human gut".

3.2.2 Likelihood of gene transfer from GM plants to animals

604. It is possible that Roundup Ready 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 Roundup Ready® canola and horizontal gene transfer is therefore possible, although unlikely.

605. 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.

606. 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.

607. Alexander et al. (Alexander et al. 2002) recently investigated the digestive fate of DNA from 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 introduced by genetic modification and an endogenous nuclear-encoded *rbcS* gene (encoding the small subunit of the photosynthetic enzyme Rubisco).

608. 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.

609. Similarly, recent studies have demonstrated that CP4 EPSPS DNA could not be detected in muscle tissue of pigs (Jennings et al. 2003b), chickens (Jennings et al. 2003a) or in milk of dairy cows (Phipps et al. 2002) fed Roundup Ready® soybean.

610. 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.

611. 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).

612. 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).

613. 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.

614. 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.

615. There is no evidence that the genes present in Roundup Ready® canola could be transferred 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

616. Transfer of the introduced genes from Roundup Ready canola® to microorganisms is extremely unlikely.

Transfer to bacteria

617. 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 1999; De Vries & Wackernagel 1998; De Vries et al. 2001) and even then only, at a very low frequency.

618. 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; De Vries & Wackernagel 1998; Nielsen et al. 2000). Without the artificially introduced homology in the recipient strain, no uptake of DNA could be detected in either *Actinobacter* sp. (Nielsen et al. 2000; De Vries et al. 2001) or *Pseudomonas stutzeri* (De Vries et al. 2001). Transformation of *Actinobacter* 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.

619. 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).

620. 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 *Actinobacter* 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).

621. Studies have identified that plant DNA survives for some time in the animal digestive tract (Duggan et al. 2000; Einspanier et al. 2001; Aumaitre 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).

622. 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 an inverse 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.

623. 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. Digestion of foreign DNA has also been identified as a barrier to horizontal gene transfer in some bacteria (Berndt et al. 2003). 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.

624. The two novel genes introduced into Roundup Ready® canola 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

625. 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

626. 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 2000c; Hodgson 2000a; Ho et al. 2000). 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

627. 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 transfer 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.

628. Both 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 Roundup Ready® canola itself.

APPENDIX 6 HERBICIDE RESISTANCE AND HERBICIDE USE

629. 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.

630. Regulation of agricultural chemicals is principally the responsibility of the Australian Pesticides and Veterinary Medicines Authority (APVMA). However, the Gene Technology Regulator is mindful of the importance of glyphosate to Australia in both agricultural and non-agricultural environments. Previous feedback from stakeholders has also raised concerns that inappropriate use of the Roundup Ready® crop - Roundup Ready® herbicide combination may lead to resistance.

631. Although the potential for herbicide resistance development is not limited to GM crops, both the OGTR and the APVMA recognise the importance of assessing potential risks associated with the use of herbicides on GM crops. Over the past year, both agencies have been consulting with a range of key stakeholders to evaluate the issues that may arise from the extended use of glyphosate.

SECTION 1 HERBICIDE RESISTANCE DEVELOPMENT

632. There is some potential for development of herbicide-resistant weeds if the Roundup Ready® crop-Roundup Ready® herbicide combination is used inappropriately. The repetitious use of a single herbicide, or herbicide group, increases the chance that selection of weeds that have developed herbicide resistance through natural mechanisms will occur (Gressel 2002).

Glyphosate

633. Glyphosate is arguably one of the most commonly applied herbicides in the world (Bayliss 2000). It is popular because it is cheap, effective and a relatively benign chemical for operators and the environment (eg Smith & Oehme 1992).

634. Data from the U.K. indicate the strong preference for glyphosate as a non-selective herbicide over the alternative broad spectrum herbicides paraquat +/- diquat. In 1998, 84 % of the cropping regions of the U.K. (878,326 ha) were sprayed with glyphosate (including pre-emergence, before harvest and other weed management scenarios) compared with 16 % (168,227 ha) sprayed with paraquat +/- diquat (pre-emergence or other scenarios) (Orson 2002).

635. Similar usage patterns of glyphosate and paraquat +/- diquat have been reported for Australia (S. Powles pers. comm.). Glyphosate has been reported as the most extensively used herbicide in Australia, with use approaching 15 000 tonnes per annum based on product sales data (Radcliffe 2002). By comparison, the annual usage of the pyridils group, of which paraquat dichloride and diquat are the primary herbicides, is reported as approximately 1000 tonnes. Glyphosate is widely used in agricultural systems in Australia to control a broad range of weeds prior to planting, and after harvest of crops as well as for general weed management around farms (*eg*. fencelines and field margins) (Dignam 2001; Neve et al. 2003b S. Powles pers comm). Glyphosate is also used for non-selective weed control in horticultural, industrial and other situations. Typical use patterns for herbicide in various canola varieties is shown in Table 1.

Table 1. Examples of typical herbicide use patterns for different canola varieties in Australia[+]

Month	Conventional canola	Triazine tolerant conventionally bred TT™ canola	Imidazolinone tolerant conventionally bred Clearfield™ canola	Glyphosate tolerant genetically modified Roundup Ready® canola	Glufosinate tolerant genetically modified InVigor® canola
November	HARVEST PREVIOUS CROP *eg.* wheat				
March-April	'knockdown' existing weeds using glyphosate or paraquat/diquat cultivate*	'knockdown' existing weeds using glyphosate or paraquat/diquat or atrazine	'knockdown' existing weeds using glyphosate or paraquat/diquat	'knockdown' existing weeds using glyphosate or paraquat/diquat	'knockdown' existing weeds using glyphosate or paraquat/diquat
April	Pre-planting weed control using trifluralin	-	Pre-planting weed control using trifluralin	Pre-planting weed control using trifluralin	Pre-planting weed control using trifluralin
	SOW CANOLA (with metalochlor in high rainfall areas)	SOW CANOLA with atrazine	SOW CANOLA	SOW CANOLA	SOW CANOLA
April-May	'pre-emergent' weed control	-	-	-	-
Early June	'post-emergent' weed control using clopyralid	'post-emergent' weed control using atrazine	'post-emergent' weed control using OnDuty® herbicide (imazapic/imazpyr)	'post-emergent' weed control with Roundup Ready® herbicide	'post-emergent' weed control with Liberty® herbicide (glufosinate-ammonium)
Late June	grass control using clethodim + haloxyfop*	grass control using clethodium*	grass control using clethodium*	grass control using Roundup Ready® herbicide*	
October	WINDROW CANOLA				
November	HARVEST CANOLA				

+ after (Norton 2003b)

* options – dependent on the variety and volume of weeds, timing of rains, soil type *etc.*

atrazine: Group C; clethodim: Group A; clopyralid: Group I; glyphosate: Group M; haloxyfop: Group A; Liberty: Group N; OnDuty: Group B; trifluralin: Group D; paraquat/diquat:: Group L; metalochlor: Group K.

636. The increased adoption of zero and minimum tillage systems (see below) in many marginal cropping areas throughout Australia, particularly Western Australia, has increased the use of non-selective herbicides, such as glyphosate and paraquat, for pre-sowing 'knock-down' weed control while simultaneously reducing soil erosion and related costs. Major disturbance of soil through tillage is decreasingly popular. With decreasing use of cultivation as a weed control strategy, glyphosate is the cheapest, and considered the most benign, of the available non-selective herbicide options. In Australia, glyphosate is the most frequently used herbicide (as much as 90%) for this purpose (Neve et al. 2003b).

Minimum tillage

637. 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 itself (zero tillage). This is in contrast to other cropping systems where the soil may be cultivated a number of times to eliminate weeds before the crop seed is sown (Anon. 2001). Significant proportions of crops in Australia are seeded using no-till methods(Sutherland 1999).

638. Since weeds are no longer controlled by non-selective tillage methods, crop rotation sequences and seeding techniques are highly dependent on the use of herbicides (Sutherland 1999). Non-selective herbicides, such as glyphosate, are commonly used in broad-acre cropping in Australia for pre-sowing 'knock-down' control of weeds. In Australia, glyphosate is by far the most frequently used herbicide (as much as 90%) for this purpose (Neve et al. 2003b). It is important to note that use of glyphosate for pre-sowing knockdown is not restricted to canola production, but to broadacre cropping in general. Therefore the APVMA's registration conditions take into account use requirements across this sector.

639. Many producers have moved from rotations including a pasture phase, to continuous cropping practices with weed control becoming more dependent on the selective herbicides 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 due to the difficulty of controlling brassicaceous weeds during cultivation. Triazine and imidazolinone tolerant canola now form a significant proportion (up to 70%) of the canola crop (Norton 2003b, refer to Appendix 5 for more details).

640. Previously canola rotations in Australia tended to be limited to about once in every four years because of presumed pathogen load of blackleg disease caused by the fungus *Leptosphaeria maculan*s (Howlett et al. 1999). However in recent times the frequency of canola in the continuous cropping rotation has increased, and in some parts of southern New South Wales it may be grown in consecutive years (Norton et al. 1999). It should be noted that a number of factors influence farmer's decisions on rotational crop choices including commodity prices, disease load and drought and it is not possible to identify one typical canola rotation that would be followed by all farmers.

Table 2. Examples of typical cropping rotations in Australia.

Year	Example 1	Example 2	Example 3	Example 4	Example 5	Example 6
1	Fallow	Pasture	Pasture	Canola	Pasture	Wheat
2	Canola	Wheat	Canola	Wheat	Canola	Canola
3	Wheat	Canola	Wheat	Wheat	Wheat	Wheat
4	Pasture/ Legume	Wheat	Wheat	Barley	Legume	Canola
5	Wheat/Barley	Pasture/ Legume	Legume	Fallow/ Legume	Wheat	Wheat

641. 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 use minimum or no till techniques for their operations.

642. A recent analysis by Norton (2003b) concluded that the adoption of GM herbicide tolerant canola varieties, such as Roundup Ready® canola, in Australia could result in a significant increase in the use of minimum tillage in canola production.

Evolution/Development of herbicide resistance

643. The evolution of resistance to herbicides is not a new phenomenon that has arisen as a result of the development of GM crops. Whenever selective pressure is applied by herbicide use the development of resistance is a possibility (Gressel 2002). However some herbicides have proven to be less prone to resistance development and some plant species also exhibit a greater ability to develop resistance to herbicides than others.

644. Each herbicide is classified into a group depending on its mode of action with each group having a different mode of action. Glyphosate is a group M herbicide and is the only group M herbicide registered by the APVMA in Australia.

645. Despite frequent and widespread use of glyphosate both in Australia and worldwide over the past 25 years, the evolution of glyphosate resistance is rare. Glyphosate is considered a low risk herbicide for the development of herbicide resistance because its mode of action imposes genetic and biochemical constraints associated with potential mechanisms of resistance (Jasieniuk 1995; Bradshaw et al. 1997). However, a number of resistant biotypes have been reported in recent times.

646. The first confirmed cases of glyphosate resistance in Australia were in populations of *Lolium rigidum* (rigid ryegrass) (Powles et al. 1998; Pratley et al. 1999). Subsequently, other resistant populations of *L. rigidum* have been verified in Australia and South Africa,

with 38 confirmed glyphosate resistant populations in Australia in 2002 (C. Preston pers. comm. September 2003).

647. The majority of these populations have developed in cropping or horticultural situations with intensive use of glyphosate, and little or no tillage. No other effective herbicides are available for control of ryegrass as many populations are also resistant to Group A herbicides (Lorraine-Colwill et al. 2002).

648. *Lolium rigidum* has developed resistance to nine different herbicide modes of action, however the propensity for resistance evolution is not the same for all modes of action (Heap 2002). Ryegrass species, including *L. rigidum*, are considered to be predisposed to the development of herbicide resistance, and the incidence of resistance to a range of other herbicides in Australian populations of *L. rigidum* is high (Heap 2003).

649. Glyphosate resistance has been reported in a number of other weed species around the world: *Conyza bonariensis* (hairy fleabane) in South Africa and North America; *Eluesine indica* (goosegrass) in Malaysia; *Lolium multiflorum* (Italian ryegrass) in Chile; and *Plantago lanceolata* (Buckhorn platain) in South Africa (Heap 2003).

Management of glyphosate resistance in Australia

650. The repetitious use of a single herbicide, or herbicide group, increases the chance that selection of weeds that have developed herbicide resistance through natural mechanisms will occur. Integrated weed management practices help to avoid selection of resistant weed biotypes (Avcare 2003). As noted above, development of resistance is considered to be most likely in *L. rigidum* populations and management strategies are available that address this issue in relation to Roundup Ready® canola (Monsanto 2003). It should be noted that the presence of resistance in *L. rigidum* tends to be geographically discrete, *ie.* often confined to particular paddocks where selection pressure through herbicide application has occurred. *L. rigidum* is a weed of agriculture and is not considered an invasive weed of undisturbed habitats.

651. Glyphosate is a widely used chemical in Australia and its use will remain high, suggesting that the number of weed populations resistant to glyphosate will continue to rise even in the absence of the introduction of Roundup Ready® crops.

652. Modelling studies, based on current usage patterns, predict that there will be widespread resistance to glyphosate in *L. rigidum* populations in zero tillage situations in Australia within 20 to 30 years (Neve et al. 2003b; Preston et al. 1999).

653. Neve et al (Neve et al. 2003a; Neve et al. 2003b) have recently developed a series of models to simulate the evolution of glyphosate resistance in *L. rigidum* over a 30 year period in a typical Australian continuous cropping rotation of wheat/lupins/wheat/canola. The models simulated the rate of development of resistance under different management strategies.

654. In situations where seeding involves full soil disturbance and glyphosate applied annually to control *L. rigidum* pre-seeding the model predicts no development of glyphosate resistance after 30 years. However, when crops were sown with minimal soil disturbance, resistance was predicted in 90% of *L. rigidum* populations after 30 years, indicating that the probability of glyphosate resistance increases in zero tillage systems. Alternating between minimum and full soil disturbance or modes of action of pre-seeding herbicides (glyphosate and paraquat) reduced the development of resistance, but did not eliminate it entirely.

655. The sequential application of two herbicides with different modes of action (the 'double knock-down') has been advocated as a mechanism to reduce the likelihood of weed populations evolving resistance to any one herbicide (Diggle et al. 2003; Neve 2003). The use of glyphosate followed by paraquat preseeding is predicted to reduce the evolution of resistance to less than 2 % of the *L. rigidum* populations.

Potential impact of Roundup Ready canola on the evolution of glyphosate resistance in Australia.

656. As outlined above, models developed in Australia to simulate the evolution of glyphosate resistance in *L. rigidum* predict that the introduction of glyphosate tolerant canola into the zero tillage cropping system currently used in Australia would significantly increase the rate at which glyphosate resistance evolves compared to rotations incorporating conventional canola varieties (Neve et al. 2003b; Preston et al. 1999).

657. Although the predicted timeframe for glyphosate resistance following the introduction of Roundup Ready® canola differs between these two models, each predicts that the evolution of resistance would be slowed by using a variety of established and currently used management techniques to maintain small population sizes of the weeds and by reducing the selection pressure. There is some evidence that Roundup Ready® canola could reduce the weed burden in subsequent rotations thereby reducing the selection pressure for herbicide resistance (Stanton et al. 2003a).

658. Herbicide resistance management strategies include the use of alternative non-selective herbicides for pre-seeding weed control thereby eliminating glyphosate application in some phases of the crop rotation, full soil disturbance at seeding and the sequential use of glyphosate followed by paraquat pre-seeding (the 'double knockdown'). Neve et al. (2003b) predict that to eliminate the increased probability of resistance with the introduction of Roundup Ready® canola, an integrated approach incorporating high crop seeding rates and removal of weed seeds at harvest combined with the 'double knockdown' preseeding would be required.

SECTION 2 HERBICIDE RESISTANCE AND THE APVMA

659. As mentioned above, the APVMA has primary regulatory responsibility for agricultural chemicals in Australia.

660. In addition to the licence granted by the Gene Technology Regulator for the commercial release of Roundup Ready® canola, the APVMA has approved a variation of the registration Roundup Ready® herbicide to enable its use on Roundup Ready® canola (APVMA 2003b). Roundup Ready® herbicide was previously registered for use only on Roundup Ready® cotton in Australia.

661. The APVMA operates the national system that evaluates, registers and regulates agricultural and veterinary chemical products. Any changes to a product that is already on the market must also be referred to the APVMA.

662. Each submission to the APVMA is evaluated to ensure that the product is safe for people, animals and the environment, that it will not pose any unacceptable risk to Australia's international trade (eg. by exceeding international residue limits) and that it will perform according to the label claims. If the APVMA is satisfied that the product meets these criteria, it may be registered for use in Australia with an APVMA approved label.

663. As part of its charter, the APVMA also manages a national compliance program in partnership with the States and Territories to ensure that products are used in accordance with their approval and their labels continue to meet the conditions of registration.

664. All submissions to the APVMA are treated on their merits and applicants are free to address any issue through the provision of data or through scientific argument. In the case of variation to the use pattern of a currently registered herbicide (*eg*. an application to extend use from non-selective ex-crop weed control to selective in-crop weed control in a commercial crop such as GM canola), applicants must specifically address how this change in use will affect the current risks, to a level acceptable to the APVMA.

665. Registrants who make submissions to the APVMA for a change in use involving a herbicide tolerant crop, are subjected to a comprehensive assessment in which they must address a variety of issues including the following:

Residues

- Will there be metabolites produced which may change the residue profile?
- Is the current maximum residue level (MRL) adequate/appropriate ?
- If the MRL is changed, how will this affect trade ?

Occupational Health & Safety

- Will the operator exposure hazard change through changes to mixing/application methods/frequency?

Efficacy & Crop Safety

- Can the herbicide be applied at all stages of crop growth ?
- At what rates can the herbicide be applied at the various stages of crop growth ?
- Will subsequent crops be affected ?
- Will any change to subsequent crops change traditional crop rotations ?
- Will efficacy be compromised through the development of premature resistance ?
- Will there be any effect on current integrated/resistance management strategies ?

Environmental Sustainability

- How will the agricultural chemical burden change ?
- What will be the likely shift in the weed spectrum ?
- How can a shift in weed spectrum be managed ?
- How will the development of weed resistance, related to increased use of the herbicide, be monitored and managed ?
- If appropriate, what are the implications of using a traditionally non-selective herbicide as a "selective" herbicide ?
- What are the options for the control of volunteer crop plants in arable and non-arable situations ?

Labelling

- What additions/changes to the label can be made to address environmental sustainability concerns for example:
    - limits on the timing and number of applications per crop;

- changes to the current resistance weeds warning;
- directing the user to Resistance Management Plans/GAP Guidelines;
- control options for volunteers; and/or
- broadening of compatibility/tank mix statements.

666. The APVMA cannot register the changed use pattern of a herbicide (*eg.* to use a previously registered herbicide on a new GM crop) before a licence is issued by the Gene Technology Regulator for that particular crop. The APVMA and the OGTR therefore work closely to ensure thorough, coordinated assessments are undertaken and, wherever possible, that the timing of assessments and decisions by both agencies coincide.

667. The Roundup Ready® (glyphosate) herbicide has been previously assessed by the APVMA for use on Roundup Ready® cotton. Herbicide resistance was identified as a key issue during that evaluation. However, it was determined that this issue could be managed through the implementation of a herbicide resistance management strategy. Roundup Ready® herbicide was subsequently registered by APVMA for use on Roundup Ready® cotton under conditions which required the implementation of a HRM plan, including ongoing reporting on compliance and effectiveness.

668. Similarly, a key element of the APVMA conditions of registration governing the extension of use on Roundup Ready® canola is the implementation of a herbicide resistance management strategy to be managed by the Herbicide Resistance Consultation Group (HRCG), which will report to the APVMA. The HRCG membership is oversighted by the APVMA and consists of representatives from NSW & Victorian State Departments of Agriculture, Co-operative Research Centre for Weeds, the Western Australian Herbicide Resistance Initiative, Charles Sturt University, Grains Research and Development Corporation, canola growers and Monsanto. Conditions have also been imposed relating to reporting of resistant weeds and auditing and reporting to the APVMA on the resistance management strategy. This strategy will complement the overall integrated weed resistance management strategies being employed within Australian cropping systems.

669. The Regulator strongly supports the APVMA imposing conditions on the application of herbicide to adequately address possible development of glyphosate resistance associated with the extension of use of the Roundup Ready® herbicide to Roundup Ready® canola.

670. The OGTR and the APVMA will continue to liaise to ensure the consistent identification, evaluation and management of risks associated with the application of agricultural chemicals to GM crops.

Monsanto's Herbicide Resistance Management Plan

671. Monsanto has developed a Roundup Ready® Canola Resistance Management Plan (RRCRMP) to reduce the likelihood of the selection of glyphosate-resistant weeds through the use of Roundup Ready® canola. The RRCRMP advocates "the strategic adoption and implementation of practical management practices within a crop rotation incorporating Roundup Ready® canola, that will manage weed populations in a manner that will ensure the long-term sustainable use of glyphosate herbicide".

672. The RRCRMP requires that growers intending to sow Roundup Ready® canola undertake a paddock-specific assessment to determine the likelihood of the weed population (in particular *L. rigidum*) developing glyphosate resistance. This assessment takes into account the previous history of herbicide use in that paddock, including the frequency and intensity of selection, and the number of herbicide modes of action to which ryegrass is resistant in the paddock proposed for sowing. This assessment provides the

basis for determining whether Roundup Ready® canola represents an appropriate crop choice, and for selecting appropriate weed management options.

673. Monsanto recommends that glyphosate not be applied in the year following Roundup Ready® canola since glyphosate would be ineffective in controlling Roundup Ready® canola volunteers. However, where this is not feasible or practical, Monsanto's RRCRMP indicates that a number of additional management practices must be implemented. Monsanto also recommends that glyphosate not be used for the control of annual ryegrass in areas where resistant populations are suspected.

674. In addition, Monsanto requires growers to sign a technology user agreement and be trained to follow the Roundup Ready® Canola Crop Management Plan (CMP). The CMP aims to ensure awareness of the industry protocols for coexistence of genetically modified and other canola and to promote knowledge of the regulatory conditions placed on the seed and herbicide.

675. The use of Roundup Ready® canola must be carefully weighed with the consequences of increased selection intensity for further weed resistance to glyphosate.

676. In order to achieve good weed control, Monsanto recommends that weed managers adopt integrated weed management strategies. The possibility of exacerbating the natural evolution of herbicide tolerance in weed species by relying on the continuous application of a single herbicide (whether glyphosate or some other) must be a consideration in the development and implementation of such weed management activities. Monsanto also recommends that rotations of other crops immediately following Roundup Ready® canola incorporate alternative herbicide management practices to glyphosate.

SECTION 3 POSSIBLE IMPLICATIONS FOR THE USE OF OTHER HERBICIDES

677. A number of submissions raised the concern that the herbicides likely to be used for the control of Roundup Ready® canola volunteers may be more toxic or more persistent than glyphosate. These herbicides could include, among others, 2,4-D, paraquat or trifluralin.

678. Such herbicides are registered for use by the APVMA (see previous section). The APVMA ensures that the use-pattern associated with these herbicides as specified by label conditions does not compromise the safety of users or the environment. The APVMA also has a program to review registered agricultural chemicals that may pose unacceptable risks to people or the environment (see below) and a program has recently been initiated for reporting any adverse effects associated with agricultural chemical use.

679. There are indications that the introduction of herbicide resistant canola in Canada has resulted in an overall decrease in herbicide usage (Brimner & Stephenson 2002) although there is some debate on this matter in other countries (Gianessi et al. 2003; Benbrook 2003). In North America the deployment of Roundup Ready® crops demonstrated environmental benefits associated with the use of glyphosate, including reduced contamination of surface water and lower levels of residual chemicals in the soil (Wauchope et al. 2002; Nelson.G.C. & Bullock 2003). It is possible that a similar effect could be observed in Australia through decreases in the use of some triazines and imidazolinones, which are currently used in conjunction with triazine tolerant ('TT' canola) and imidazolinone tolerant canola (Clearfield®, 'IT' or 'IMI') respectively (Norton 2003b). Atrazine, the most widely used triazine herbicide in Australia, is a common contaminant of Australian surface waters where it is generally below the threshold for ecological effects (NRA 2002). It is also often found in groundwater aquifers at low levels. Atrazine is currently under review by the APVMA.

680. Roundup Ready® canola can offer another weed management tool for farmers that will widen the choice of strategies available for weed control and containment of herbicide resistance in Australian farming systems.

681. ARMCANZ endorsed a National Strategy for Agricultural and Veterinary Chemicals in 1998 (ARMCANZ 1998). The intent of the Strategy is to "maximise benefits from the use of agvet chemicals while minimising the risks of undesirable side-effects". The Strategies objectives include reduced reliance on chemicals – encourage integrated pest management, reduced handling and environmental risks - best management practices, monitor and assess outcomes of chemical use. The suite of regulatory controls and stewardship initiatives afforded by Government, Monsanto and industry that will underpin the introduction of Roundup Ready canola to the Australian cropping system also include measure to achieve integrated weed management.

SECTION 4 CONCLUSIONS REGARDING HERBICIDE RESISTANCE AND CHANGED USE OF OTHER HERBICIDES

682. There is potential for development of herbicide-resistant weeds if the Roundup Ready® crop-Roundup Ready® herbicide combination is used inappropriately. The development of resistance to glyphosate does not pose risks to human health and safety or the environment. However, it would have implications for the *choice* of herbicide(s) available for weed control operations in agriculture and elsewhere. The APVMA assesses all herbicides used in Australia for safety and sets their conditions of use. The APVMA can also review registration of herbicides. For example, the herbicide 2, 4-D (one of the most commonly used tank-mix herbicides) and atrazine are currently under review.

683. Herbicide resistance is managed by the APVMA, under conditions of registration for the use of agricultural chemicals in Australia. Therefore the Regulator has not imposed any specific licence conditions in relation to management of herbicide resistance for this release. However the assessment and imposition of conditions by the APVMA to address the management of herbicide resistance is strongly supported. The APVMA and OGTR have reporting requirements for applicants and will continue to have an oversight role with Roundup Ready® herbicide and Roundup Ready® canola respectively.

APPENDIX 7 INDUSTRY GUIDANCE MATERIAL

SECTION 1 INDUSTRY AND GOVERNMENT REPORTS

684. 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 implications, market impacts and cost/benefit issues have been intentionally excluded from the *Gene Technology Act 2000* assessment process. Such issues were excluded because concerns were raised during the extensive consultation process that led to the development of the legislation, that a requirement for the Regulator to consider economic issues might compromise the focus of the regulatory system upon the scientific evaluation of risks and the protection 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 associated with the commercial introduction of GM canola to farmers or the agricultural industry.

685. However, these issues are being actively considered by the Australian, 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 *Roundup Ready® Canola Crop Management Plan* were both considered during the evaluation process to identify any additional risks in relation to the proposed commercial release that may arise from proposals they contained.

686. Both documents relate to procedures to segregate GM and non-GM canola to the extent required by markets. 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 does not anticipate any containment measures, such as buffer zones (*ie*. the risk assessment process considered the risks that might occur in the absence of supply chain management controls). The key elements of these documents are outlined in Section 2 & 3 below.

687. There are a number of reports concerning the potential consequences of the introduction of GM canola on markets, including:

- the Productivity Commission report *Modelling Possible Impacts of GM Crops on Australian Trade*
- the Australian Bureau of Agricultural & Resource Economics (ABARE) report *Australian Grains Industry 2003-GM Canola. What are its economics under Australian conditions?*
- the ABARE report *Market Access Issues for GM Products – Implications for Australia*

688. There are also a number of studies, commissioned by the Australian Government Department of Agriculture, Fisheries and Forestry relating to the Agricultural Biotechnology Projects: Supply Chain Management for GM Products.

- The Bureau of Rural Science (BRS) study *Gene Flow Study – Implications for the Release of GM Crops in Australia*
- The BRS study – *Agricultural Biotechnology: Herbicide Tolerant Crops in Australia*

- The Australian Government Analytical Laboratories (AGAL) study – *Review of Technologies for Detecting GM Materials in Commodities and Food*
- The Tasmanian Quality Assured Incorporated study – *Gap Analysis in relation to Quality Management for the Supply Chain Management of GM products*.

689. There are a number of industry funded documents that provide background on canola production in Australia for both conventional and GM canola, including:

- The Australian Oilseed Federation report *Genetically Modified Canola in Australia* prepared by Phil Salisbury at the University of Melbourne
- The Avcare report *Conservation Farming Systems and Canola* prepared by Robert Norton at the University of Melbourne

1. In addition, the Victorian Government has appointed Professor Peter Lloyd from the University of Melbourne to make an independent study of the issues surrounding GM-canola, other canola crops and overseas trade. A report is expected in early 2004.

690. From an international perspective some recent reports include:

- the ABARE report *Agricultural Biotechnology: Potential for use in developing countries*
- The Danish Institute of Agricultural Sciences (DIAS, Ministry of Food, Agriculture and Fisheries, Denmark) hosted the 1st European Conference on the Co-existence of Genetically Modified Crops with Conventional and Organic Crops in November 2003. The abstracts from this meeting including a number of papers on canola

SECTION 2 GENE TECHNOLOGY GRAINS COMMITTEE

691. 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 produced a discussion paper on a strategic framework for co-existence in the canola industry that was released to the public on 1 August 2002 for comment by the end of September 2002. The final strategic framework for maintaining coexistence of supply chains framework was released in December 2002

692. The Canola Industry Stewardship Principles (CISP) for Coexistence of Production Systems and Supply Chains (Gene Technology Grains Committee 2003) were released for extensive consultation. The Eastern and Western zones of the GTGC adopted a number of resolutions in relation to the CISP in October 2003. These were that the GTGC:

- acknowledge the CISP form a basis for the continued development of a dynamic process towards the management of coexistence;
- support the formation of an Australian Oilseeds Federation sponsored, expertise-based Canola Reference Group.

2. The Canola Reference Group will make recommendations to the GTGC regarding the ongoing development of the CISP and assume responsibility for the communication, monitoring and reporting of their implementation.
3. The CISP describe various mechanisms by which all participants in the production and processing supply chain for canola can achieve 'coexistence' between GM and non-GM canola production systems.

4. They 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. The various components of the supply chain are detailed in Figure 1.

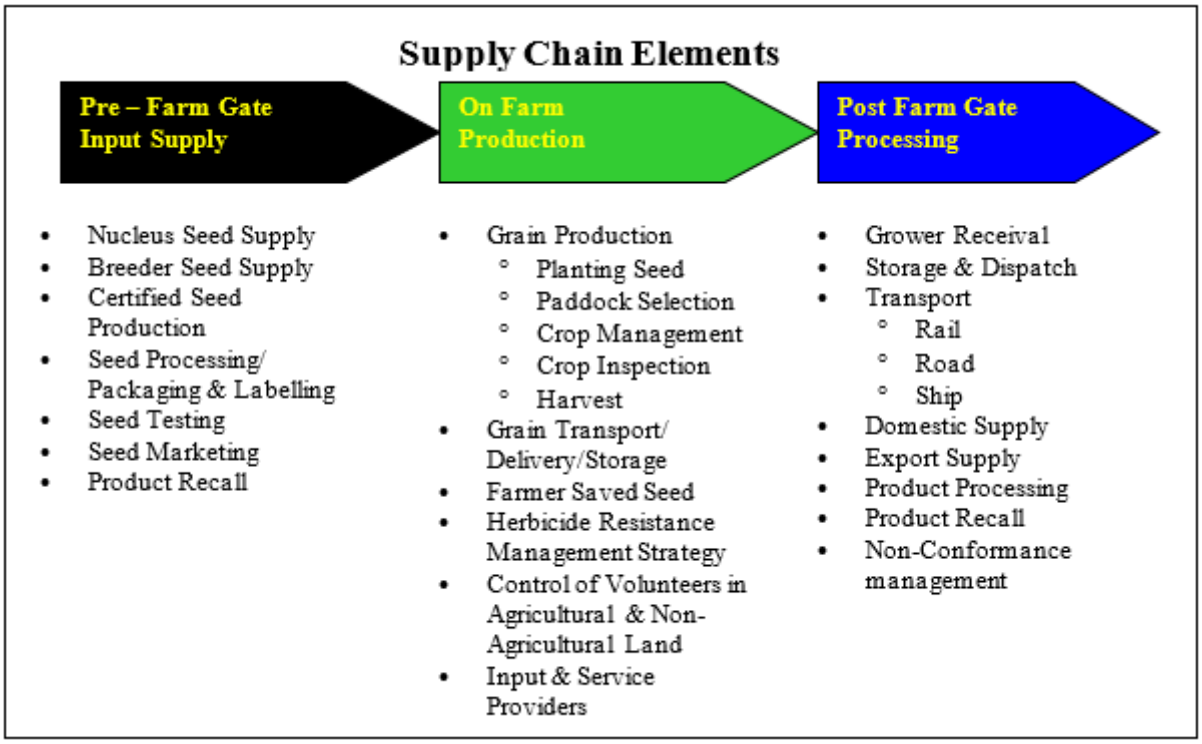

Figure 1 Diagrammatic presentation of canola supply chain elements (adapted from GTGC Canola Industry Stewardship Principles (2003))

SECTION 3 MONSANTO'S STEWARDSHIP STRATEGY

693. In accordance with the GTGC guidelines, Monsanto has developed a stewardship strategy for Roundup Ready ® canola, which is underpinned by a number of key systems and documents, including the *Roundup Ready® Canola Crop Management Plan, Roundup Ready® Herbicide Resistance Management Plan* and *Roundup Ready® Canola Technical Manual*.

694. During the assessment Monsanto submitted its draft *Roundup Ready® Canola Crop Management Plan* and associated documents. These were declared 'Confidential Commercial Information' under section 185 of the Act. The documents were draft versions that could not be finalised until regulatory approvals were received from the Regulator and the APVMA. Monsanto has indicated that these documents will be finalised and released in the near future (refer www.monsanto.com.au).

695. In accordance with section 184 of the Act this information was not available to the general public. However the information was available to the expert groups that are required to be consulted on the preparation of the RARMP.

696. The stated aims of the Roundup Ready ® Canola stewardship strategy are to:

- Ensure compliance with regulatory requirements;
- Allow the co-existence of different canola production systems;

- Achieve the sustainability of both Roundup Ready® technology and glyphosate herbicide; and
- Enable growers to maximise the overall benefit from the technology.

697. Monsanto identifies several critical components for the successful implementation of the Roundup Ready® canola stewardship strategy, including:

- Standards for managing the technology (government, industry and Monsanto);
- Communication and training, including an Accreditation program for growers;
- Auditing and compliance; and
- Reporting deviations from standards and any adverse events.

698. Other key systems and documents include:

- Roundup Ready® Canola Technical Manual;
- Roundup Ready® Canola Herbicide Resistance Management Plan, including the Paddock Risk Assessment Management Option Guide (PRAMOG);
- 'Roundup Ready® Herbicide by Monsanto' Label and Directions for Use;
- Training for growers, including an Accreditation Program and support from In-field Service Providers;
- Monsanto Biotechnology Management Manual; and
- Roundup Ready® Canola Technology User Agreement;

699. Under their stewardship strategy, Monsanto proposes to educate all growers and agronomists/resellers about the standards for managing the technology.

700. Monsanto will require growers of Roundup Ready® canola to implement on-farm practices that aim to:

- Prevent the evolution of herbicide resistant weeds;
- Control Roundup Ready® canola volunteers;
- Minimise risks to the integrity of grain supply chains;
- Ensure good crop agronomy in a sustainable manner; and
- Meet all other regulatory requirements.

701. 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 Roundup Ready ® canola. Monsanto 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.

5. To ensure compliance with the guidelines, Monsanto proposes to audit growers, seed companies and seed distributors. Monsanto's target number of audits for the first 4 years following approval are provided in Table 2.

Table 1: The minimum number of grower audits proposed by Monsanto during first 3 seasons following approval (assuming approval is granted in 2003)

		Minimum % of growers audited		
Audit	Main Requirements	2003	2004	2005+
Post Herbicide Application	Ensure weed survey undertaken, surviving weeds reported, paddock records maintained	100%	10%	5%
Grain Delivery	Ensure grower declarations accurate	0%*	5%	5%
Seed Storage	Ensure farmer saved seed managed correctly	10%	10%	5%
Following Season	Ensure Resistance and Crop Management Plans implemented	100%	10%	5%
Seed Production	Ensure quality assurance for seed production followed	100%	100%	100%
Seed Lot Distribution	Ensure recording of seed lots and suppliers that Roundup Ready® canola seed has been sold to	100%	50%	50%
Seed Distribution to Growers	Ensure recording of seed lots and growers that Roundup Ready® canola seed has been sold to	50%	25%	10%

* Monsanto will work closely with growers and grain handlers in the first year to ensure effectiveness of grain delivery processes.

+ Criteria for 2005 and subsequent years will be reviewed at that time.

SECTION 4 SEED PRODUCTION IN AUSTRALIA

702. Until recently the Commonwealth Government delegated the operational functions of the OECD Seed Certification Scheme to a number of organisations, mainly State and Territory departments. In February 2003 the operational aspects of the OECD Seed Certification Scheme in Australia were delegated to the industry owned Australian Seeds Authority (ASA) (ASA 2003). Via this delegation, ASA performs the functions of Australia's National Designated Authority under the Organisation for Economic Co-operation and Development (OECD) Seed Schemes and Designated Authority under the International Seed Testing Association (ISTA) (ASA 2003).

703. The ASA is comprised of a membership of the Seed Industry Association of Australia (SIAA) and the Grains Council of Australia.

704. The SIAA has a national code of practice for labelling of seed for sowing and marketing (SIAA 1999) and is currently developing an national industry standard for the adventitious presence of genetically modified seed in Australian seed lots. This standard has not yet been finalised but will be determined on the basis of meeting domestic and international seed and bulk commodity market requirements.

705. Further details on the ASA, SIAA, seed production and seed certification can be obtained from the SIAA website: http://www.sia.asn.au

706. As described in Appendix 5, isolation requirements for certified seed production in Australia are based on OECD Seed Certification Scheme - Rules and Directives (OECD 2003; Glover 2002), however individual proprietary users (seed producers) will implement measures based on their own quality assurance and control needs. Industry standards for isolation and quality assurance relating to production and marketing of seed for sowing will reduce the likelihood of outcrossing resulting in glyphosate tolerant 'off types' in non-Roundup Ready® canola seed lots.

707. Monsanto has indicated that the production of Roundup Ready® canola seed for distribution to growers will be conducted according to the industry standards for the production of certified canola seed, and that strict quality assurance protocols will be followed. Roundup Ready® canola seed production plots for breeders' seed will be isolated from other canola crops by a minimum distance of 400 m. These measures will minimise the level of contamination of Roundup Ready® canola seed by surrounding canola crops and also limit the potential for gene transfer to occur from Roundup Ready® canola seed production plots to surrounding canola crops.

APPENDIX 8 PROPOSED LICENCE CONDITIONS AND REASONS FOR THE CONDITIONS

Gene Technology Regulation in Australia

The Gene Technology Act (2000) and corresponding State and Territory legislation form an integral part of a range of regulatory measures which control the development and use of genetically modified organisms (GMOs) in Australia.

The Gene Technology Regulator is required to consult with, and takes into account advice from, a range of regulatory authorities on risks to human health and safety and the environment in assessing applications for dealings involving the intentional release of GMOs into the Australian environment.

Note continuing operation of other State laws relating to GMOs

This licence does not authorise dealings with GMOs that are otherwise prohibited by State laws that declare GM, non-GM free zones or both for marketing purposes. These laws are administered and enforced by the States and Territories.

Note in relation to herbicide resistance management

The GMO referred to in this licence has been modified to be tolerant to a herbicide. The APVMA has responsibility for setting registration conditions for the use of herbicides in Australia, including implementation of herbicide resistance management programs. Conditions of this licence do not relate to use of herbicide, and do not displace any conditions set by the APVMA.

SECTION 1 GENERAL CONDITIONS

Duration of Licence

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

Holder of Licence

2. The holder of this licence ('the licence holder') is Monsanto Australia Ltd.

Project Supervisor

3. The Project Supervisor in respect of this licence is identified at Attachment A.

4. The licence holder must immediately notify the Regulator in writing if any of the contact details of the Project Supervisor change.

No dealings with GMO except as authorised by this licence

5. Persons covered by this licence must not deal with the GMO except as expressly permitted by this licence.

GMO covered by this licence

6. The GMO covered by this licence is described at Attachment B.

Permitted dealings

7. The permitted dealings with the GMO are all dealings with the GMO.

Persons covered by this GMO licence

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

Informing people of their obligations

9. The Licence holder must inform any person covered by this licence, to whom a particular condition of this licence applies, of the following:

(a) the particular condition (including any variations of it);
(b) the cancellation or suspension of the licence;
(c) the surrender of the licence.

Licence holder to notify of circumstances that might affect suitability

10. 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 Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment;
(c) any event or circumstances occurring after the commencement of this licence that would affect the capacity of the holder of his licence to meet the conditions in it.

Additional information to be given to the Regulator

11. It is a condition of a licence that the Licence holder inform the Regulator if the licence holder:

(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.

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

12. If a person is authorised by this licence to deal with 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.

Remaining an accredited organisation

13. The licence holder must, at all times, remain an accredited organisation in accordance with the Act and comply with its instrument of accreditation.

SECTION 2 INTERPRETATION AND DEFINITIONS

In this licence:

Words and phrases used in this licence have the same meaning as they do in the Act and the Regulations;

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 and equivalent provisions, if any, in corresponding State law, 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 has a corresponding meaning;

Specific conditions prevail over standard conditions to the extent of any inconsistency.

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

'GM' means genetically modified.

'GMO' means the genetically modified organism covered by this licence described at Attachment B.

'OGTR' means the Office of the Gene Technology Regulator.

'Project Supervisor' means the person identified as the Project Supervisor at Attachment A.

'Regulator' means the Gene Technology Regulator.

SECTION 3 SPECIFIC CONDITIONS

Testing Methodology

1. 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 GMO 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

2. Each year, the licence holder must prepare a written annual report on the administration of the licence for the previous year.

3. The period for an annual report is the year ending on anniversary of the day this licence is issued.

4. 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.

5. 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 GMO or material from the GMO;
(b) Information about the volumes of the GMO 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 the GMO 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 GMO, including annual surveys, the details of which will be determined in consultation with the OGTR.

Note: Attachments A & B are included with the licence.

SECTION 3 REASONS FOR LICENCE CONDITIONS

General licence conditions

The general licence conditions in Section 1 of the licence restate the statutory licence conditions that apply to the licence.

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 conditions 2-5 require information about the quantities of the GMO 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.

APPENDIX 9 LEGISLATIVE REQUIREMENTS FOR ASSESSING DEALINGS INVOLVING INTENTIONAL RELEASES

SECTION 1 THE REGULATION OF GENE TECHNOLOGY IN AUSTRALIA

708. The *Gene Technology Act 2000* (the Act) took effect on 21 June 2001. The Act, supported by the *Gene Technology Regulations 2001*, 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).

709. The Act establishes a statutory officer, the Gene Technology Regulator (the Regulator), to administer the legislation and make decisions under the legislation.

710. The Regulator is supported by the Office of the Gene Technology Regulator (OGTR), a Commonwealth regulatory agency located within the Health and Ageing portfolio.

711. 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).

712. The requirements under the legislation for consultation and for considering and assessing licence applications and preparing risk assessment and risk management plans (RARMPs) are discussed in detail in Division 4, Part 5 of the Act and summarised below.

713. 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

714. Licence applications for dealings involving the intentional release (DIR) of a genetically modified organism into the environment 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.

715. The application must also contain:

- 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;

➢ supporting information from the Institutional Biosafety Committee.

716. 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.

717. 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

718. In accordance with Section 50 of the Act, the Regulator must seek advice in preparing a 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.

719. 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 national and regional news papers, in the *Gazette* and on the OGTR website) in respect of the application, inviting written submissions on whether the licence should be issued.

720. 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. Copies of applications are available on request from the OGTR.

SECTION 4 THE EVALUATION PROCESSES

721. The risk assessment process is carried out in accordance with the *Act* and *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.

722. 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.

723. In considering this information and preparing the RARMP, 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 a GMO;
- the effect, or the expected effect, of the genetic modification that has occurred 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.

724. 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

725. Having prepared a risk assessment and a risk management plan, the Regulator must, under Section 52 of the Act, seek comment from stakeholders, including those outlined in Section 3 and the public.

726. All issues relating to the protection of human health and safety and the environment raised in written submissions on an application or a risk assessment and a risk management plan 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.

727. Comments received in written submissions on this RARMP are very important in shaping the final RARMP and in informing the Regulator's 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 RARMP.

728. 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

729. 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 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.

730. 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.

731. The Regulator carefully considers all of this information which is supplied in a declaration signed by licence applicants.

732. 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.

733. If a licence is issued, the Regulator may impose licence conditions (Section 62 of the Act) to manage risks posed to the health and safety of people, or to the environment. For example, conditions may be imposed to:

- limit the scope of the dealings;
- require documentation and record-keeping;
- require a level of containment;
- limit the dissemination or persistence of the GMO or its genetic material in the environment;
- specify waste disposal methods;
- require data collection, including studies to be conducted;
- limit the geographic area in which the dealings may occur; and
- require contingency planning in respect of unintended effects of the dealings.

734. 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 of the Act). 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 of the Act). 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.

735. 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 unexpected problems.

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

OVERVIEW

Input from the public, interested organisation and government agencies has provided valuable feedback. Public feedback is an essential ingredient in Australia's gene technology regulatory scheme. Public consultation helps ensure that issues can be raised and risks investigated to determine whether or not the risks can be managed. A discussion of the issues raised in consultation on this Risk Assessment and Risk Management Plan (RARMP) is provided in this Appendix.

Comments on the RARMP were wide ranging – from philosophical objections to gene technology generally, through to support for this canola variety in particular. Submissions ranged in length and substance from short one-sentence comments through to detailed papers covering many pages.

All of these comments were read by OGTR. Many of the issues raised were taken into account in the preparation of the consultation version of the RARMP. However, the consultation comments highlighted areas that required further explanation and we have sought to do this as part of this final package.

14. The OGTR received 94 written submissions from individuals and organisations during the public consultation process on the RARMP.

15. A total of 58 campaign letters and e-mails (four types in all were received) and one (1) petition was received with 12 signatures. 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.

16. A total of eleven (11) types of issue were raised which can be categorised into three broad groups:

- issues dealing with the genetic modification(s);
- issues which are the responsibility of other agencies; and
- issues which fall outside the consideration of the Gene Technology Regulator and other associated agencies.

DETAILED CONSIDERATION OF ISSUES

17. 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 concerning the genetic modification(s)

18. This includes matters related to the protection of human health and safety and the environment and also the suitability of Monsanto Pty Ltd to hold a licence in accordance with section 58 of the Act.

19. 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 revised and expanded to further explain the evaluation process and the basis of the conclusions reached as follows:

Issue	Enhanced explanation
1. General Health concerns	see Appendix 2
2. Precaution and general safety	see Appendix 9
3. General environmental concerns	see Appendices 3, 4 and 5
4. Pollen flow and contamination	see Appendices 5 and 7
5. Gene transfer to weeds	see Appendix 5
6. Applicant suitability	see Chapter 2 Appendix 9

Issues which are the responsibility of other agencies

20. Many submissions raised issues that related to matters that are the responsibility of either Agricultural Pesticides and Veterinary Medicines Authority (APVMA), 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, which is responsible for food safety and labelling, including GM foods.

21. This group of issues comprises the following categories :

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

22. Although the regulatory responsibility rests with the APVMA, Issue 7 has been discussed in Appendices 6 and 7.

Issues which fall outside the consideration of the Gene Technology Regulator and other regulatory agencies

23. 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.

24. 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.

25. Comments on these issues were considered, but as they are outside the scope of the Act, no assessment has been made for the purposes of the RAMP. However, the RARMP does have some discussion of these issues in the sections indicated:

Issue	Enhanced explanation
9. Agricultural practices	see Appendices4 and 6
10. Economic/market issues	see Chapter 2 and Appendix 7

11. Other general issues	

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