



Office of the Gene Technology Regulator

APPLICATION FOR LICENCE FOR INTENTIONAL RELEASE OF A GMO INTO THE ENVIRONMENT:

Application No. DIR 021/2002

SUMMARY INFORMATION

Updated April 2003

Project Title:	Commercial release of genetically modified canola (<i>Brassica napus</i>) for use in the Australian cropping system
Applicant:	Bayer CropScience Pty Ltd* 391-393 Tooronga Rd East Hawthorn VIC 3123 *Formerly Aventis CropScience Pty Ltd.
Common name of the parent organism:	Canola
Scientific name of the parent organism:	<i>Brassica napus</i>
Modified traits:	Hybrid breeding system, herbicide tolerance and antibiotic resistance (not in lines proposed for commercial release)
Identity of the genes responsible for the modified traits:	<i>Bar</i> gene from the bacterium <i>Streptomyces hygroscopicus</i> (herbicide tolerance) <i>pat</i> gene from the bacterium <i>Streptomyces viridichromogenes</i> (herbicide tolerance) <i>barnase</i> gene from the bacterium <i>Bacillus amyloliquefaciens</i> (male sterility, hybrid breeding system) <i>barstar</i> gene also derived from <i>B. amyloliquefaciens</i> (fertility restorer, hybrid breeding system) <i>nptII</i> gene from the bacterium <i>Escherichia coli</i> in some lines (antibiotic resistance)
Proposed Location	Potentially all canola growing regions of Australia. NB The growing of genetically modified food crops in Tasmania would also require approval by the Tasmanian State Government under the <i>Plant Quarantine Act 1997</i> .
Proposed Size of Release:	Small scale first year introduction in south-east Australia, up to full commercial release in all canola growing regions.
Proposed Date of Release:	From Autumn 2003

Bayer is seeking regulatory approval for seven similar genetically modified ‘lines’¹ of canola: *T45*, *Topas 19/2*, *MS1*, *RF1*, *RF2*, *RF3* and *MS8*. Lines *MS1*, *MS8*, *RF1*, *RF2* and *RF3* and hybrids derived from MS x RF crosses are covered by the registered trade name InVigor[®] canola.

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

The proposed dealings

Bayer seeks approval for the commercial release of its GM canola in all canola growing regions of Australia and continued product development and research programs. Proposed areas of the release include all Australian States and Territories.

It is intended that Bayer’s GM canola plants and their by-products would be used in the same manner as conventional canola. Canola is primarily grown for its seeds, which yield oil and high protein animal feed. Canola oil is used in a variety of products including low-fat foods, pharmaceuticals, margarine, nutritional supplements and salad dressings. During the processing of (GM and non-GM) canola oil, DNA and introduced proteins are removed. The oil derived from all seven lines has been approved by Food Standards Australia New Zealand (FSANZ, formerly the Australia New Zealand Food Authority, ANZFA) for human consumption.

Parent organism

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>).

Genetic modification and its effects

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

The genetic modifications introduced into the male sterile and fertility restorer lines of InVigor[®] canola enable a breeding system for the production of hybrid canola seed. Hybrid canola varieties produced using conventional (non-GM) breeding techniques have also been developed. Non-GM hybrid canola is estimated to represent about 6% of the Australian canola market. Bayer’s GM canola lines also confer resistance to the herbicide glufosinate

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

ammonium. Non-GM triazine and imidazolinone tolerant canola varieties currently comprise approximately 60% of the Australian canola market.

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

The development of the pollen producing parts of canola flowers (anthers) is suppressed in MS plants. Crossing an MS line with an RF line overrides the suppression and makes the progeny fertile. The progeny are expected to have enhanced agronomic performance, otherwise known as ‘hybrid vigour’. A more detailed explanation of this ‘hybrid vigour’, which is also utilised in conventional breeding, is provided in Appendix 1 of the risk assessment and risk management plan that can be obtained from the Office of the Gene Technology Regulator or from the Office’s website at www.ogtr.gov.au.)

Table 1: Genetic modifications in Bayer canola lines

GM canola Line	Glufosinate ammonium tolerance	Hybrid breeding system	Antibiotic resistance
T45	<i>Pat</i>	-	-
Topas 19/2	<i>Pat</i>	-	<i>NptII</i>
MS1	<i>Bar</i>	<i>barnase</i>	<i>NptII</i>
MS8*	<i>Bar</i>	<i>barnase</i>	-
RF1	<i>Bar</i>	<i>barstar</i>	<i>NptII</i>
RF2	<i>Bar</i>	<i>barstar</i>	<i>NptII</i>
RF3*	<i>Bar</i>	<i>barstar</i>	-

* Lines proposed to be commercialised in Australia

The male sterility gene (*barnase*) and a fertility restorer gene (*barstar*), are both derived from *Bacillus amyloliquefaciens*, a common soil bacterium. Both genes are linked to a herbicide tolerance gene: the *bar* gene. The *bar* gene, derived from a soil bacterium *Streptomyces hygroscopicus*, codes for the enzyme phosphinothricin acetyl transferase (PAT) which detoxifies phosphinothricin (glufosinate-ammonium), the active ingredient in the herbicide Liberty®.

An antibiotic resistance gene (*npt II*) has been transferred into lines Topas 19/2, MS1, RF1 and RF2. This gene is derived from a bacterium *E.coli* and codes for an enzyme neomycin phosphotransferase which detoxifies antibiotics such as kanamycin and neomycin, thereby conferring resistance to the bacteria in which the recombinant plasmids are maintained. This is mainly used a selectable marker for the early selection of transformed plants in tissue culture.

Short regulatory sequences that control expression of the genes are also present in the genetically modified canola. These sequences are derived from the cauliflower mosaic virus, *Agrobacterium tumefaciens*, *Arabidopsis thaliana* and *Nicotiana tabacum*. Although the first

two organisms are plant pathogens, the regulatory sequences comprise only a small part of their total genome and are not in themselves capable of causing disease.

Detailed information on the *bar*, *pat*, *barnase*, *barstar* and *nptII* genes, and characterisation of the inserted genetic material and the new proteins expressed by InVigor® canola are provided in Appendix 1 of the risk assessment and risk management plan.

Table 2: Genetic elements and their origin.

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

Method of gene transfer

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

Previous releases and international approvals

Previous Australian Releases

A number of limited and controlled releases (field trials) have been previously approved to assess the agronomic performance of all seven lines and their behaviour in the Australian environment. Fourteen limited and controlled releases of the lines were conducted under the voluntary system overseen by GMAC, as listed below:

- Male sterile and fertility restorer lines (RF1, RF2, RF3, MS1 and MS8), including the resultant hybrids such as InVigor® (MS8 x RF3), PR-63, PR-63X, PR-63X(2), PR-63X(3), PR-63X(4), PR-63X(5), PR-63X(6) and PR-63X(7), and
- Glufosinate-ammonium tolerant open-pollinated lines T45 and Topas 19/2 PR-62, PR-62X, PR-62X(2), PR-62X(3), PR-62X(4) and PR-62X(5).

The first release in Australia of lines covered by this application was in 1996. All previous releases have been carried out under conditions to limit spread or persistence of the GMO in the environment. The lines have been grown in various Australian locations and conditions in New South Wales, Victoria, South Australia, Western Australia, Queensland and Tasmania to select the best varieties for further development. In the largest approved trial, the planting

area was 712 hectares. No adverse effects on human health and safety or the environment were reported for any of these releases.

On 30 July 2002 the Regulator issued a licence (DIR 010/2001) to Bayer for a limited and controlled release of InVigor[®] canola on 90 sites in 23 shires in New South Wales, Victoria, Western Australia and South Australia comprising a total area of 318 hectares over 3 years (106 hectares per annum).

The approvals issued by GMAC and the Regulator included conditions for the management of the trials to minimise the risks posed by the GM canola. Monitoring undertaken by the IOGTR identified a number of instances of non-compliance with GMAC conditions, as detailed in IOGTR Quarterly Reports. Some of these instances of non-compliance related to trials of InVigor[®] canola, specifically PR-63X(4) and PR-62X(4). In some of these instances Bayer notified the IOGTR of the non-compliance. No instances of non-compliance were subsequently identified by the IOGTR or the OGTR for the reporting periods April-June 2001 and July-September 2001. There were no instances of non-compliance in the periods October-December 2001, January-March 2002, April-June 2002, and July-September 2002.

Most of the instances of non-compliance related to post-harvest monitoring licence conditions, in particular the requirement to remove volunteer canola from the trial site prior to flowering. In each instance GMAC and the IOGTR/OGTR assessed the risks posed to human health and safety or the environment as a result of the non-compliances as negligible. Bayer also undertook management actions to further minimise any risks, including the removal of volunteers, destruction of the current crop on the site and extension of the monitoring period for non-compliant sites.

Studies commissioned by the OGTR on gene flow from non-compliant sites did not demonstrate any gene flow to other Brassicaceous species. There have been no observed adverse effects on human health and safety or the environment from these incidents.

Organisations are also required to provide monthly monitoring data to the OGTR. In October and November 2002 Bayer provided a number of monitoring reports to the OGTR in regard to former GM canola sites in Tasmania at which flowering volunteers had been identified. The sites of concern were: PR-63X(4), Site 73 PR-62X(4), Site 14 PR-62X(4), Site 13. These sites were in post harvest monitoring phase, ie the GM canola had been harvested and 'post-harvest' crops (not canola) had been sown.

A risk assessment conducted by the OGTR determined that at two of these sites detection of volunteer GMO canola was difficult due to the cover crops and could lead to a risk of persistence of the GMO in the environment and its possible dissemination. Bayer arranged to destroy the post harvest crops at the two sites to allow for control of volunteer plants. The OGTR determined that continued monitoring by Bayer at the remaining site would allow for adequate control of GM volunteer canola growth.

Approvals by Other Australian Government Agencies

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

Food Standards Australia New Zealand (FSANZ)

The safety and labelling of foods derived from genetically modified plants are the responsibility of FSANZ, rather than the OGTR.

Only canola oil is consumed by humans in Australia (OGTR, 2002). FSANZ (formerly ANZFA) have approved the use of oil derived from the glufosinate-ammonium tolerant male sterile, fertility restorer and resultant hybrid lines for use in food in Australia. FSANZ has determined that refined oil derived from these lines of canola is as safe for human consumption as refined oil derived from conventional canola (non-GM) varieties (see Appendix 2 of the risk assessment and risk management plan).

Further details of the risk analysis conducted by FSANZ on lines *T45*, *Topas 19/2*, *MS1*, *RF1*, *RF2*, *RF3* and *MS8* and information about food labelling are available from FSANZ:

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

Australian Pesticides and Veterinary Medicines Authority (APVMA)

The registration of herbicides is the responsibility of the Australian Pesticides and Veterinary Medicines Authority (APVMA), formerly known as the National Registration Authority (NRA), rather than the OGTR.

Glufosinate ammonium is not registered for use in broad-acre cropping in Australia. It is a limited-use herbicide that is most commonly used as Basta[®] for weed control in horticultural crops. Bayer is seeking registration of glufosinate ammonium for use on InVigor[®] canola under the trade name Liberty[®]. APVMA is considering this application. If registered by APVMA, Liberty[®] would only be used on InVigor[®] canola crops, not for weed control in other crops. Appendix 4 of the risk assessment and risk management plan contains further details.

Further information about the use and safety of insecticides and herbicides can be obtained from:

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

International Approvals for the seven canola lines

The seven lines included in the application lines have been approved for food (Table 1), feed (Table 2) and environmental (Table 3) safety in a number of countries.

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

Country	Event	Year Approved
Australia	MS1/RF1/RF2/MS8/RF3	2002
Australia	T45	2002
Australia	Topas 19/2	2002
Belgium	1. MS1/RF1	1995
Canada	MS1/RF1	1994
Canada	MS1/RF2	1995
Canada	MS8/RF3	1997
Canada	T45	1997
Canada	Topas 19/2	1995
Europe	MS8/RF3	Submitted
Japan	MS1/RF1	1996
Japan	MS1/RF1/RF2	1997
Japan	MS8/RF3	1997
Japan	T45	1997
UK	MS1/RF1	1995
UK	MS1/RF2	1995
UK	Topas 19/2	1995
USA	MS1/RF1	1996
USA	MS1/RF2	1996
USA	MS8/RF3	1998
USA	T45	1997
USA	Topas 19/2	1995

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

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

USA	MS1/RF2	1996
USA	MS8/RF3	1998
USA	T45	1997
USA	Topas 19/2	1995

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

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

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

The Bayer canola lines MS8 and RF3, and their hybrid (line MS8 x RF3) have been approved for growing and consumption in the US, Canada and Japan and their environmental and food safety are currently being assessed by regulators in Europe. The Scientific Committee on Plants of the European Commission concluded that InVigor[®] canola was unlikely to cause adverse effects on human health and the environment.

The Belgian Government has refused to approve an application from Bayer to conduct field tests with GM herbicide tolerant canola. In the communique on the decision, the Belgian Minister noted that pollen may be transferred up to 4 km by bees and that there was therefore a chance of dissemination of the GMO even with containment measures and that in their assessment there was uncertainty regarding possible effects on the environment. The issue of pollen transfer is considered in detail in Appendix 5 of the risk assessment and risk management plan. Subsequent advice from the Service of Biosafety and Biotechnology indicated that the minister's decision included considerations of adventitious presence of GM canola in the surrounding farms or in honey and isolation measures towards nature reserves.

No other country is known to have refused an application for the release of glufosinate ammonium tolerant male sterile, fertility restorer and the hybrid lines on the basis of risks to human health and safety or the environment.

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