GENE TECHNOLOGY TECHNICAL ADVISORY COMMITTEE

COMMUNIQUE
No. 12

This is the twelfth communique of the Gene Technology Technical Advisory Committee (GTTAC). It covers matters considered at the twentieth meeting of GTTAC, held on 27 April 2004 and at a teleconference held on 30 April 2004.

GTTAC is a statutory advisory committee to the Gene Technology Regulator (the Regulator) and the Gene Technology Ministerial Council. All Committee members and expert advisers hold office on a part-time basis.

The Regulator receives input from GTTAC on applications for licences to conduct dealings with genetically modified organisms (GMOs), as well as comments on the Risk Assessment and Risk Management Plan (RARMP) that is prepared for each of these applications.

The purpose of this Communique is to provide a brief overview of the applications and RARMPs considered by GTTAC and the advice the Committee has provided to the Regulator with regard to those applications and RARMPs.

The Communique also provides an overview of any other major issues discussed by GTTAC.

Dealings Not Involving the Intentional Release of Genetically Modified Organisms

Dealings Not Involving the Intentional Release of GMOs (DNIRs) are dealings that are usually undertaken within a certified facility (so that the organism is physically contained) and where the personnel involved in the dealing have been assessed as having adequate training and experience for the task. These are typically laboratory-based projects.

Applications and RARMPs for the following DNIRs were assessed:

Application Number and Title	Project Description	GTTAC Comments
DNIR 287/2004 Subcellular trafficking of the *Dengue virus* NS5 protein.	The aim of this project is to examine subcellular trafficking of the *Dengue virus* type 2 (Den-2) nonstructural protein 5 (NS5) in mammalian and insect cells and to study its role during infection by making targeted mutations within the NS5 protein sequence.	GTTAC agreed that the risk assessment identified all the risks associated with the proposed dealings and that the measures proposed in the risk management plan are adequate to deal with the identified risks. The Committee further recommended that information should be sought from the applicant regarding the facility containing the confocal microscope and if the GMO will need to be transported between laboratories.
DNIR 288/2004 Cell lines expressing *Hepatitis B virus*	The aim of this project is to study the expression, formation and release of *Hepatitis B virus* in hepatocyte (liver) cell lines, including the expression of drug-resistance genes.	GTTAC agreed that the risk assessment identified all the risks associated with the proposed dealings and that the measures proposed in the risk management plan are adequate to deal with the identified risks. The Committee further recommended that the final risk assessment should indicate that the virus is able to replicate in cell cultures but is not able to reproduce a productive infection and that the virus can survive for a comparatively long time in the environment.
DNIR 289/2004 Asexual genetic exchange in *Rhynchosporium secalis*, the causal agent of barely scald.	The aim of this project is to determine if asexual genetic exchange can occur between genetically distinct isolates of *R. secalis* and whether this exchange can result in a heritable change to the genotype of either isolate.	The Committee concluded that the risk of unintentional release was very low and that it could be managed by the containment and work practices of PC2 level laboratory facilities.

DNIR 291/2004 Analysis of *cytomegalovirus* (CMV) genes involved in antiviral susceptibility, replication and cell tropism.	The aim of this dealing is to characterise the regions of CMV genes that are required for DNA replication, antiviral susceptibility and cell tropism and to develop strategies for the inhibition of viral replication.	The Committee agreed that the risk assessment identified all the risks associated with the proposed dealings and that the measures proposed in the risk management plan are adequate to deal with the identified risks. The Committee further recommended that all people undertaking the dealing should be informed that the dealing poses increased risk to immunocompromised people.

Dealings Involving the Intentional Release of Genetically Modified Organisms

Dealings Involving the Intentional Release of GMOs (DIRs) are dealings that are undertaken outside of a contained facility. DIRs involve the limited and controlled release (field trial) of a GMO or a commercial (general) release of a GMO.

RARMPs for licence applications for DIRs are released for public comment as part of the consultation process for these applications. Information on how to obtain copies of applications and RARMPs for DIRs is provided at the end of this document.

Advice on RARMPs

Advice on Cotton

GTTAC considered the RARMPs prepared in response to the following applications concerning the release of transgenic cotton in Australia.

- **Field trial to assess transgenic cotton expressing natural plant genes for insect control (DIR 048/2003)**

The OGTR has received an application from Hexima Limited (Hexima) for the intentional release of genetically modified (GM) cotton (Hexima cotton) into the environment, on a limited scale and under controlled conditions. Hexima proposes to trial the GM cotton on a maximum of 2 sites selected from 3 shires (Wambo, Pittsworth and Jondaryan) totalling an area less that 0.5 hectares per season in Queensland during the 2004/5, 2005/6 and 2006/7 cotton-growing seasons.

The aims of the proposed release are to test the efficacy of various GM cotton lines containing plant-derived protease inhibitor (PI) genes against insect pests of cotton. Seed would also be retained for future research and possibly subsequent trial seasons for the proposed release. None of the cotton plants from the release, or their by-products, would be used for human food or animal feed, nor would lint or linters be sold for processing.

Four types of GM cottons containing plant-derived PIs are proposed for release. Two types of GM cotton contain serine PI genes (*NaPI*) derived from the ornamental tobacco *Nicotiana alata*. *NaPI*s code for precursor proteins that are processed in the plant cell to produce small proteins that act as serine PIs and as such inhibit trypsin and chymotrypsin. The third type of GM cotton contains the *PotI* gene derived from potato, *Solanum tuberosum*. *PotI* codes for a serine PI that inhibits chymotrypsin in the midgut of cotton-feeding insect larvae. The fourth type of GM cotton also contains plant-derived serine PI genes, however their identity has been declared Confidential Commercial Information (CCI) under section 185 of the Act. This information has been made available to GTTAC and other prescribed expert authorities that are being consulted on the preparation of the RARMP. The genetic modification in the fourth type of GM cotton also confers insect resistance.

All the GMOs also contain the neomycin phosphotransferase (*nptII*) *gene* from the common bacterium *Escherichia coli*, which confers antibiotic resistance to aminoglycoside antibiotics such as kanamycin. The enzyme neomycin phosphotransferase (NPTII) was used to select plants in the laboratory. NPTII is one of the most widely used selectable markers for plant transformation.

The regulatory sequences for the introduced PI genes comprise the 35S viral promoter derived from the Cauliflower Mosaic Virus (CaMV), and terminator signals from the *nos* gene from *Agrobacterium tumefaciens*, while for *nptII* both the promoter and the terminator were also derived from the *nos* gene.

The mode of action of the plant-derived PIs in Hexima cotton differs from that of bacterial Bt toxins (Cry and VIP) in previously released genetically modified Bt cottons.

GTTAC discussed this application and advised the Regulator that the following issues should be considered in the preparation of the RARMP:

- The Committee agreed with the risk assessment and the proposed licence conditions outlined in the draft RARMP prepared by the OGTR;
- Information regarding the serine PI expression levels in pollen and nectar should be sought from the applicant;
- NaPI is not widespread in the environment and that this matter should be noted in the RARMP summary table; and
- The applicant be required to gather data on toxicity for non-target organisms if a licence is issued.
- GTTAC agreed with the proposal that native bees should be protected by isolating the trial from specified habitats.

- **Agronomic assessment and seed increase of GM cottons expressing insecticidal genes (*cry1Fa* and *cry1Ac*) from *Bacillus thuringiensis* (DIR044/2003)**

The OGTR has received an application from Dow AgroSciences Australia Limited for the limited and controlled release of GM cotton containing insecticidal genes (chimeric *cry1A*c and *cry1Fa*) toxic to lepidopteran caterpillar pests of cotton, and a herbicide tolerance marker gene (*pat*). The small scale trial is proposed to occur on a total of up to 10 hectares over two summer and two winter cotton growing seasons (May 2004 – May 2006) in cotton growing regions of Queensland (Qld), New South Wales (NSW) and in the Northern Territory.

The aims of the proposed release are to test the efficacy of the two-gene insecticidal cotton line (Widestrike™) against lepidopteran caterpillar pests of cotton as compared to its two parental lines, one introducing the chimeric *cry1Fa* gene or the other introducing the chimeric *cry1Ac* gene, and to evaluate their respective agronomic performance in a range of Australian cotton growing regions. All three lines contain a herbicide tolerance marker gene that confers tolerance to the herbicide glufosinate ammonium.

The applicant also aims to collect data to develop insect resistance management plans. In addition, the applicant intends to measure the expression levels of the insecticidal proteins in cotton leaves and roots and residues of these proteins in soil, as well as to test the effect of GM cotton lines on non-target organisms. Seed would also be retained for potential future releases. None of the cotton plants from the release, or their by-products, would be used for animal feed or human food.

GTTAC discussed this application and advised the Regulator the following:

- The names of chimeric protein be indicated with an asterisk;
- GTTAC agrees with the assessment made by the OGTR on risk of toxicity, allergenicity, weediness and gene transfer; and
- GTTAC agrees with the proposed licence conditions.

Advice on Clover

GTTAC considered the following RARMP prepared in response to the following application concerning the release of transgenic clover in Australia.

- **Field evaluation of genetically modified white clover resistant to infection by alfalfa mosaic (DIR 047/2003)**

The OGTR has received an application from the Department of Primary Industries (DPI) (Victoria) for a licence for the intentional release of a genetically modified (GM) virus resistant white clover derived from a single transformation event into the environment, on a limited scale and under controlled conditions.

The applicant proposes to carry out the field trial of the GM white clover over four planting seasons between May 2004 and April 2007 on one site in Victoria, on a total area of two hectares. The site consists of a 26 x 19 metre GM white clover plot surrounded by a 32 metre wide (minimum) pollen trap of non-GM clovers and other legumes.

The main aims of the proposed release are the field evaluation of a GM white clover resistant to infection by AMV and the production of GM white clover seed for future trials, subject to further approvals. DPI (Victoria) proposes to evaluate agronomic characteristics and resistance to AMV of the GM white clover over two years and then produce seed from a selection of GM white clover plants showing superior agronomic performance and AMV resistance.

The GM white clover proposed for release contains two genes, the coat protein gene from Alfalfa mosaic virus (AMV CP) and the neomycin phosphotransferase type II (nptII) selectable marker gene from Escherichia coli.

The AMV CP gene confers resistance to infection by AMV. AMV is a single stranded RNA virus and belongs to the genus Alfamovirus, family Bromoviridae and is widespread in the environment.

The nptII gene confers resistance to aminoglycoside antibiotics related to kanamycin and neomycin.

Short regulatory sequences (promoters and terminators) that control the expression of the introduced genes are also present in the GM white clover. The AMV CP gene is under the control of the enhanced cauliflower mosaic virus 35S promoter (CaMV 35S) and the pea ribulose-1,5-bisphosphate carboxylase small subunit gene (rbcS-E9) terminator. The nptII gene is controlled by the nopaline synthase (nos) gene promoter and the terminator from the common soil bacterium *Agrobacterium tumefaciens*.

None of the white clover plants from the release, or their by-products, would be used for animal feed or human food.

GTTAC discussed the application and advised the Regulator that the following issues should be considered in the preparation of the RARMP:

- The proposed dealing involves an as yet undetermined risk posed by potential low level gene transfer;
- At this stage, the dealing should not proceed unless it is contained within a bee-proof enclosure; and
- To promote germination of seed, the release site should be ploughed to a depth of 50mm.

Advice on Applications

Advice on Cotton

- **GM Cotton Field Trial – Evaluation under field conditions of the cotton rubisco small subunit promoter driving a reporter gene (DIR 049/2004)**

The OGTR has received a licence application from CSIRO for the intentional release of genetically modified (GM) cottons into the environment, on a limited scale and under controlled conditions. The proposed release would be conducted on one site covering an area of up to 0.1 hectares in each of the summer growing seasons of 2005 and 2006 at the Australian Cotton Research Institute (ACRI) in the shire of Narrabri, New South Wales.

The aim of the proposed release is to test the efficacy of the photosynthetic promoter (*rbcS* or rubisco small subunit promoter) from cotton controlling the expression of a reporter gene (*uidA* or β-glucuronidase gene) derived from a common gut bacterium (*Escherichia coli*).

CSIRO proposes to conduct a limited and controlled release of 60 GM cotton lines to compare the performance of two different promoters – the *rbcS* promoter from cotton (30 lines) and a commonly used viral promoter, *35S* from Cauliflower Mosaic virus (30 lines). The GM cotton lines also contain either one or two antibiotic resistance genes (*hph* and/or

nptII) that were used as selectable markers during development of the GM cottons in the laboratory.

There have been no previous applications to release GM cotton lines containing the *rbcS* promoter. However, various combinations of the *35S* promoter and the introduced genes have previously been approved for release. CSIRO conducted a limited and controlled release of GM cotton containing the *uidA* reporter gene controlled by the *35S* viral promoter (PR100 and PR100X) under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC). Licences for the intentional release of GM cottons containing the *uidA* reporter gene controlled by the *35S* viral promoter, and the *nptII* and *hph* antibiotic resistance marker genes, have been issued under the current regulatory system, as listed in the table below.

Introduced Genes	DIR reference	Applicant	Type of release
35S/uidA/nptII	005/2001	Cotton Seed Distributors Ltd	Limited and controlled
	006/2001	CSIRO	Limited and controlled
	009/2001	Department of Agriculture WA	Limited and controlled
	012/2001	Monsanto	Commercial
nptII	008/2001	Department of Agriculture WA	Limited and controlled
	022/2002	Monsanto	Commercial
	023/2002	Monsanto	Commercial
hph	017/2002	CSIRO	Limited and controlled
	025/2002	CSIRO	Limited and controlled
	034/2003	Syngenta	Limited and controlled
	036/2003	CSIRO	Limited and controlled

The *35S* viral promoter was also used to control the expression of other genes such as *cry1Ab*, *cry1Ac, cry2Ab, bar* and *cp4 epsps* in GM cottons approved under DIR 015/2002, DIR 016/2002, DIR 035/2003, DIR 036/2003 and DIR 038/2003 for limited and controlled releases.

None of the cotton plants from the release, or their by-products, would be used for animal and human food. However, the applicant proposes to sell lint from the release and from surrounding pollen trap cotton plants. Lint does not contain genetic material or protein.

GTTAC discussed this application and advised the Regulator that the following issues should be considered in preparation for the RARMP:

- The risks posed by DIR 049/2004 are similar to those posed by previous cotton applications; and

- Advice in relation to previously assessed GM cottons should be considered in the preparation of the RARMP for DIR 049/2004.

Gene Technology Ethics Committee (GTEC) Paper

The Committee was asked to comment on a paper prepared by a GTEC working group, titled *Managing Risk Ethically.* The paper aims to make recommendations to improve the risk assessment process and foster trust in the regulatory system through improving the

communication of risk. The Committee discussed the paper, including its recommendations, and provided comments for the final version of the document. Further information concerning the operations of the GTEC is available on the OGTR website.

Presentations

The following presentations were made to GTTAC:

- Review of the Risk Analysis Framework;
- Interactions between regulatory agencies; and
- Overview of the Australian Pesticide and Veterinary Medicines Authority (APVMA).

Review of the Gene Technology Regulations 2001 (Regulations)

The Committee was advised that the review of the Regulations was progressing. The Committee noted the progress and agreed to forward comments on this matter to OGTR.

Enquiries and Risk Assessment and Risk Management Plans

For all enquiries and to obtain copies of applications or RARMPs for dealings involving the intentional release of GMOs into the environment, please phone the OGTR Free-call hotline on 1800 181 030. The RARMPs are also available electronically from our website at http://www.ogtr.gov.au/publications/riskassessments.htm.