GENE TECHNOLOGY TECHNICAL ADVISORY COMMITTEE

COMMUNIQUE
No. 13

This is the thirteenth communiqué of the Gene Technology Technical Advisory Committee (GTTAC). It covers matters considered at the twenty first meeting of GTTAC, held on 22 July 2004, and the twenty second meeting of GTTAC, held on 21 September 2004 via teleconference.

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 considered:

Application Number and Title	Project Description
DNIR 292/2004 Kunjin replicon virus like particles for delivery of cytokines into mice.	The aim of this project is to determine whether Kunjin replicon virus-like particles (KUN VLPs) can be used to deliver immuno-modulatory genes as a potential treatment for cancer or to prevent transplant rejection.
DNIR 293/2004 Viral delivery of genes or siRNA involved in adipogenesis or insulin signalling to cells.	The aim of this project is to examine the effect of increasing or reducing the expression of factors involved in the body's response to insulin and in human fat tissue development in mammalian cells.
DNIR 295/2004 Somatic cell genetic studies of mitochondrial respiratory chain disorders.	The aim of this project is to extend the lifespan of cultured human fibroblasts and introduce individual chromosomes into the fibroblasts in order to map the chromosomal location of genes involved in respiratory chain disorders.
DNIR 297/2004 Development of in vitro liver stage drug susceptibility assays for *Plasmodium vivax, P. falciparum, P. yoelii* and *P. cynomogli.*	The aim of this dealing is to develop *in vitro* liver stage drug susceptibility assays for malaria parasites.
DNIR 298/2004 A Phase I/IIa, two-centre, open label, dose escalation study to assess the safety, tolerability and efficacy of FP253 in combination with fludarabine phosphate.	The aim of this project is to assess the safety, tolerability and efficacy of a candidate cancer therapeutic in a Phase I/IIa clinical trial in prostate cancer patients
DNIR 299/2004 Characterisation of replication competent hepatitis B viruses (HBV).	The aim of this project is to express complete genomes of cloned hepatitis B viruses (HBVs) from penguins and bats in cultured eukaryotic cells *in vitro* to test viral replication, and to test the ability of resulting HBV particles to infect primary host cells in cell culture.
DNIR 302/2004 Generation of stable cell lines expressing Hepatitis B Virus using the ViraPower lentiviral expression system.	The aim of this study is to express the human Hepatitis B virus (HBV) genome in human cultured cells using a HIV-1-based lentiviral expression system and to characterise HBV synthesis in stable human cell cultures.
DNIR 305/2004 Wnt/FZSD in human cancer	The proposed dealings aim to determine the role of FZD and Wnt genes, in particular FZD7 (frizzled Drosophila homolog 7), in the morphological changes that lead to metastasis of colon tumour cells.

Application Number and Title	Project Description
DNIR 307/2004 Molecular studies of human immunodeficiency virus (HIV-1) and hepatitis C virus (HCV)	The proposed dealings aim to study the fusion and entry of HIV-1 and (HCV) in human cell lines in order to develop antiviral therapeutics and vaccines targeting virus-cell interactions.

GTTAC agreed that the risk assessments for the proposed dealings identified all risks associated with human health and safety and the environment, and that the measures proposed in the risk management plan are adequate to deal with the identified risks.

The Committee further agreed that, in relation to DNIR 305/2004, caution should be used by those conducting the dealings when handling sharps.

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 include limited and controlled releases (eg: field trials) and commercial releases of GMOs.

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 genetically modified cotton

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

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

The OGTR has received an application from CSIRO for the intentional release of genetically modified (GM) cottons into the environment, on a limited scale and under controlled conditions. CSIRO proposes to conduct a small scale, limited and controlled release of 60 GM cotton lines on one site covering an area of up to 0.1 hectares in each summer growing season at the Australian Cotton Research Institute (ACRI) in the Shire of Narrabri, NSW. The release is planned for October 2004 to May 2006 and encompasses two summer growing seasons.

The aim of the proposed release is to evaluate the efficacy of the rubisco small subunit (*rbcS*) promoter, compared to the *35S* viral promoter in controlling the expression of the *uidA* reporter gene in the GM cotton lines under Australian field conditions.

The GM cotton lines covered by this application are for research purposes only and are still in the early development stages. The antibiotic resistance marker genes (*nptII* and *hph*), the reporter gene (*uidA*), the viral promoter (*35S*) have been used in a number of GM cottons that have previously been approved for intentional release (see 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

However, this is the first application for a licence involving the expression of an introduced gene under the control of the *rbcS* promoter from cotton.

GTTAC discussed this application from CSIRO and advised the Regulator that:

- GTTAC agreed with the assessment made by the OGTR on risk of toxicity, allergenicity, weediness and gene transfer; and

- GTTAC agreed with the proposed licence conditions.

Advice on Applications

Advice on genetically modified bovine herpesvirus 1 vaccine

- **Vaccination of cattle with recombinant bovine herpesvirus 1 vaccines (DIR 050/2004)**

The OGTR has received an application from the Queensland Department of Primary Industries and Fisheries for a licence for the intentional release of genetically modified (GM) BoHV-1 vaccines into the environment on a limited scale and under controlled conditions within Physical Containment Level 1 (PC1) animal containment facilities which are sufficient to contain cattle. The applicant has proposed that the releases take place between October 2004 and 2009. The unmodified BoHV-1 (serotype 1.2b) is found in cattle and buffalo populations all over the world, including Australia. BoHV-1 serotype 1.2b is the only BoHV-1 serotype that has been found in Australia. Up to 180 cattle will be involved in this trial.

The proposed trial involves the use of four GMOs based on BoHV-1 that have been modified by the addition of full length (E2) or truncated (E0) genes from bovine viral diarrhoea virus (BVDV)and/or by the addition of a gene encoding the GFP marker gene (see Table 1). The inserted genes will be under the control of the human cytomegalovirus immediate early (hCMV ie) promoter. The BVDV E2 gene encodes the major target of neutralising antibodies

against BVDV infection. E2, in conjunction with E0 and other BVDV proteins that will not be used in this dealing, are the determinants of BVDV host range. Green fluorescent protein (GFP) will also used in the constructs as a novel foreign antigen to allow serological differentiation of vaccinated animals from those with a natural BoHV-1 infection.

Immunisation with the GM vaccines will be used to determine:

- the immune response of the vaccinated cattle to the BoHV-1, BVDV and GFP antigens;
- the ability of the GM vaccines to protect cattle from a challenge with BoHV-1 or BVDV or a bacterial pathogen; and
- the influence of pre-existing immunity to BoHV-1 and BVDV on the efficacy of the BoHV 1 vaccines.

Table 1 GMOs proposed for release under DIR 050/2004

GMO	Parent virus	Promoter	Gene inserted	Poly-A
1	BoHV-1	hCMV-ie	GFP	Rabbit β-globin poly-A
2	BoHV-1	hCMV-ie	E2	Rabbit β-globin poly-A
3	BoHV-1	hCMV-ie	E0-GFP	Rabbit β-globin poly-A
4	BoHV-1	hCMV-ie	E2 + E0-GFP	Rabbit β-globin poly-A

The applicant sought approval to declare details of the insertion sites of the BVDV antigens as confidential commercial information (CCI). This information has been declared CCI.

GTTAC discussed this application and advised the Regulator that the following issues should be considered:

- the species specificity of the parent virus;
- the role that the inserted genes may play in the host range of the recombinant virus;
- the potential for the GM BoHV-1 to be more toxic or allergenic to cattle than the parent organism;
- any effect that the inserted genes may have on the lifecycle of the GM virus including the ability to be re-activated following a latent infection;
- whether the proposed containment is adequate; and
- whether Food Standards Australian New Zealand (FSANZ) has approved any products containing GFP for human consumption.

The OGTR advised GTTAC at their 22nd meeting that the clock has been stopped on this application since the applicant has advised that other BoHV-1 GMOs are likely to be added to the application and that the use of GFP as a marker is being reviewed.

Advice on genetically modified sugarcane

- **Field trial of sugarcane expressing sucrose isomerase (DIR 051/2004)**

The OGTR has received an application from the University of Queensland for a licence for the intentional release of genetically modified (GM) sugarcane into the environment on a limited scale and under controlled conditions at 2 sites covering maximum total area of 3.55 ha in the Burdekin Shire, Queensland. The applicant has proposed that the release will occur between early 2005 and late 2010. Plantings are proposed to take place during March-May and August-October in each of 2005, 2006 and 2007.
The proposed trials involve up to 120 transgenic lines of GM sugarcane containing the sucrose isomerase (*si*) gene isolated from the bacterium *Panthoea dispersa* and the aminoglycoside resistance gene (*aphA* or *nptII*) from the bacterium *Escherichia coli* as a selectable marker. The *si* gene transferred into the GM sugarcane confers upon the plant the ability to express the sucrose isomerase enzyme which converts sucrose into its isomer isomaltulose.

The aims of the proposed release are to:

- determine the agronomic performance of the GM sugarcane lines under field conditions including concentrations of different sugars in various tissues over the growing season; and

- observe the presence of any indirect effects caused by the genetic modifications eg. alteration of sensitivity to environment and biological stress.

The results will be used to guide the experimental adjustment of parameters such as timing and strength of expression of the introduced genes, for optimal beneficial effects in sugarcane improvement.

GTTAC discussed this application and advised the Regulator that the following issues should be considered in relation to this application:

- potential for toxicity/allergenicity of GM sugarcane to humans and other organisms;

- the potential for GM sugarcane to be harmful to the environment because of an increased potential for weediness;

- potential for gene transfer posed by the release of these GMOs and its consequences; and

- obtain further useful information from the Bureau of Sugarcane Experiment Stations (BSES) and sugarcane experts regarding destruction of sugarcane after harvest.

Advice on genetically modified rice

- **Phenotyping of T-DNA and/or transposon *Ds* insertion line of rice (*Oryza sativa* L.) under field conditions (DIR 052/2004)**

The OGTR has received an application from CSIRO for a licence for the intentional release of genetically modified (GM) rice (*Oryza sativa* L. cv Nipponbare) into the environment, on a limited scale and under controlled conditions.

CSIRO proposes to carry out the release at one site in the local government area of Wagga Wagga City Council, NSW over three growing seasons between October 2004 and May 2008, including provision for one fallow season if required. However, the statutory timeframe for consideration of the application extends until February 2005. Therefore if a licence were to be issued it would be likely to cover the growing seasons between 2005 and 2009.

The aims of the proposed release are:

- to identify rice genes influencing traits of biological or agronomic interest by observing alterations in the visible characteristics (phenotypes) of GM rice lines which were generated under contained (laboratory and glasshouse) conditions; and

- to characterise gene flow in rice under Australian field conditions.

The proposed trial involves the planting of approximately 1500 different GM rice lines (usually 30 plants of each line). The lines contain various combinations of commonly used reporter genes and antibiotic resistance and herbicide tolerance genes as selectable markers, as well as transposable *Ds* elements and 'plasmid rescue' elements.

GTTAC discussed this application from CSIRO and advised the Regulator that the following issues should be considered:

- the potential for GM rice to be harmful to humans or other organisms because it may be toxic or allergenic;

- the potential for GM rice to be harmful to the environment because of inherent weediness or increased potential for weediness and the potential for 'shattering' to lead to a persistent seedbank;

- the potential for the new genes introduced into the GM rice to transfer to other organisms with adverse consequences;

- whether the 150m isolation zone was sufficient, considering seed movement by rodents;

- seek further information from the applicant concerning the proposed 2km distance from breeding lines; and

- monitoring of the proposal at the end of three plantings.

Advice on genetically modified wheat

- **Field testing of salt tolerant wheat on saline land (DIR 053/2004)**

The OGTR has received an application from Grain Biotech Australia Pty Ltd (GBA) for a licence to intentionally release genetically modified (GM) salt tolerant wheat (*Triticum aestivum* L.) on a limited scale under controlled conditions. The proposed release would take place in Corrigin shire, Western Australia on 0.45 ha from April 2005 to April 2006. The aim of the proposed release is to evaluate the salt tolerance and agronomic performance of GM salt-tolerant wheat in a field affected by different levels of salinity.

The GM salt tolerant wheat has been genetically modified to contain the ornithine amino transferase gene (*oat*) isolated from a common plant, *Arabidopsis thaliana*. The *oat* gene produces the enzyme, ornithine amino transferase enzyme (OAT). Over-expression of this enzyme can increase proline levels in the plant. The GM wheat also contains the selective marker gene, cyanamide hydratase (*cah*) isolated from the soil fungus *Myrothecium verrucaria*. The *cah* gene produces the enzyme cyanamide hydratase (CAH) that confers cyanamide resistance by hydrating the nitrile group of cyanamide to produce urea.

The proposed release would consist of the GM wheat, non-GM bread wheat, a non-GM barley, a non-GM durum wheat and non-GM salt adapted bread wheat.

None of the material harvested from the trial, including seed will be used for human food or animal feed. Any material not used for research will be destroyed.

GTTAC discussed this application from Grain Biotech Australia and advised the Regulator that the following issues should be considered:

- The potential for toxicity/allergenicity of GM wheat to humans and other organisms;
- The potential for GM wheat to be harmful to the environment because of an increased potential for weediness; and
- The potential for gene transfer posed by the release of these GMOs and the consequences of such gene transfer.

- **Field trial – Alteration of grain starch in wheat (DIR 054/2004)**

The OGTR has received an application from CSIRO Plant Industry (CSIRO) for a licence to allow the intentional release of genetically modified (GM) wheat into the environment on a limited scale and under controlled conditions. The release is proposed to take place at one site covering a maximum total area of 0.05 ha in the Australian Capital Territory (ACT) from May 2005 to December 2006. The aim of the proposed release is to assess the field performance of GM wheat with altered starch characteristics and to generate seed stocks of the wheat lines for future research.

CSIRO has sought and received approval to have details of the gene constructs, sequence information, and precise identification of the genes involved declared as confidential commercial information (CCI).

The proposed trial involves six transgenic lines of the GM starch-altered wheat. Gene silencing (RNAi) has been used to knockout the expression of two 'starch enzymes' (SE). The sixth line represents a vector-only transformed line as a control. All SE sequences were derived from wheat (*Triticum aestivum* L.). All lines proposed for release also contain the commonly used bacterial selectable marker gene neomycin phosphotransferase (*nptII*) from *Escherichia coli* that confers resistance to antibiotic karamycin.

GTTAC discussed this application from CSIRO Plant Industry and advised the Regulator that the following issues should be considered:

- The potential for toxicity/allergenicity of GM wheat to humans and other organisms;
- The potential for GM wheat to be harmful to the environment because of an increased potential for weediness; and
- The potential for gene transfer posed by the release of these GMOs and the consequences of such gene transfer.

Advice on genetically modified cotton

- **Field trials of herbicide tolerant (Roundup Ready® Mon 88913) and herbicide tolerant/insect resistant (Roundup Ready® Mon 88913/Bollgard II®) cotton (DIR 055/2004)**

The OGTR has received an application from Monsanto Australia Limited (Monsanto) for a licence for the intentional release of genetically modified (GM) herbicide tolerant cotton (Roundup Ready® Flex MON 88913) and herbicide tolerant/insect resistant cotton (Roundup Ready® Flex MON 88913/Bollgard II®) into the environment, under limited and controlled conditions.

Monsanto proposes to carry out field trials covering an area of up to 2011 hectares over two planting seasons, the southern summer growing season and the northern winter growing season, between September 2005 and November 2006. The summer trials would be conducted in the cotton growing regions of NSW and southern Qld, and the winter trials in northern WA, the NT and northern Qld.

The aims of the proposed release are to:

- incorporate the Roundup Ready® Flex MON 88913 (RR Flex cotton) trait into elite cotton varieties suitable for use under Australian conditions;
- test agronomic performance including disease resistance (bacterial blight, fusarium and verticillium wilt);
- produce seed for future release;
- set up demonstration sites for industry, government, researchers and the wider community; and

- collect data required for future applications to the OGTR and other regulators for commercial release such as levels of novel protein expression and seed composition (required by the OGTR and FSANZ) and data on the GM cottons' tolerance to glyphosate, weed control and glyphosate residue levels (required by the Australian Pesticides and Veterinary Medicines Authority (APVMA)).

None of the cotton plants from the release, or their by-products, would be used for animal and human food. The applicant proposes to sell lint from the release for use as fibre in the textile industry. Lint does not contain genetic material or protein.

Details of the gene construct, including the plasmid map and some of the regulatory sequences have previously been declared as Confidential Commercial Information (CCI) under section 185 of the Act, in connection with licence application DIR 035/2003.

GTTAC discussed this application from Monsanto Australia Ltd and advised the Regulator that the following issues should be considered:

- the risks posed by the proposed release under DIR 055/2004 are similar to those posed by the previous RR Flex and RR Flex/Bollgard II® cotton application (DIR 035/2003);

- the risks posed by the proposed release are also similar to those posed by the previous Roundup Ready® and Roundup Ready®/Bollgard II® cotton applications (DIR 012/2002 and DIR 023/2002); and

- advice provided in relation to the same GMOs previously assessed under DIR 035/2003 and to similar previously assessed GM cottons (Bollgard II® and Roundup Ready® cottons) should be considered in the preparation of the RARMP for DIR 055/2004.

- **Commercial release of herbicide tolerant (glufosinate ammonium) cotton (DIR 056/2004)**

The OGTR has received an application from Bayer CropScience Pty Ltd (Bayer) for a licence to intentionally release genetically modified (GM) herbicide tolerant cotton (LLCotton25) into the environment. The aim of the proposed release is to commercially release LLCotton25 into the Australian agricultural system, and undertake ongoing product research and development.

No specific containment measures have been proposed and Bayer intends that the GM cotton plants and their products would be used in the same manner as conventional and other GM cotton. Hence, the dealings would include use in human food (subject to approval by FSANZ), transportation and use as stockfeed anywhere in Australia, sale of lint and exporting seed.

LLCotton25 contains the *bar* gene which confers tolerance to the herbicide glufosinate ammonium (also called phosphinothricin), the active constituent of the herbicides Basta®, Finale®, Buster® and Liberty®. LLCotton25 plants can be sprayed with glufosinate ammonium to kill problem weeds without damaging the crop itself.

Bayer requests approval to commercially plant LLCotton25 wherever conditions are suitable for cotton cultivation. The applicant anticipates a phased introduction over 3 years, involving large scale grower evaluations and seed increases, and the development of additional lines adapted for particular regional conditions. Initially, Bayer expects the most substantial

adoption of the GM cotton to occur in the existing cotton growing regions of New South Wales and Queensland, followed by uptake in potential future cotton growing areas in these states, the Northern Territory, four shires in Western Australia, and two shires close to the NSW border in both South Australia and Victoria. Small scale use for demonstrations and educational purposes is also proposed outside these areas.

Bayer currently has a research permit from the Australian Pesticides and Veterinary Medicines Authority (APVMA) for small scale use of glufosinate ammonium on the GM cotton, and intends to submit an application to the APVMA to register the herbicide for commercial scale use.

GTTAC discussed this application from Bayer CropScience Pty Ltd and advised the Regulator that the following issues should be considered:

- the risks associated with toxicity, allergenicity, weediness or gene flow in relation to commercial scale release of LLCotton25 are negligible; and

- advice provided in relation to previously assessed GM cottons should be considered in the preparation of the RARMP for DIR 056/2004.

Presentations

The following presentations were made to GTTAC:

- Review of the Risk Analysis Framework; and

- Update on the research project on the environmental impact of GM cotton.

Review of the *Gene Technology Regulations 2001* (the 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