

# **Risk Assessment and Risk Management Plan**

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

**DIR 039/2003**

Title: Field evaluation of genetically modified high oleic cotton  
with modified fatty acid desaturase and antibiotic  
resistance genes

**Applicant: CSIRO**

**October 2003**



Office of the  
**Gene Technology Regulator**

## Abbreviations

ACRI	Australian Cotton Research Institute
ANZFA	Australia New Zealand Food Authority (now FSANZ)
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DIR	dealing involving intentional release
DNA	deoxyribonucleic acid
DNIR	dealing not involving intentional release
EMBL	European Molecular Biology Laboratory
FAD	fatty acid desaturase
FAO	Food and Agriculture Organisation of the United Nations
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
g	gram
gh	<i>Gossypium hirsutum</i> , cultivated cotton
GM	genetically modified
GMAC	Genetic Manipulation Advisory Committee
GMO	genetically modified organism
GRAS	Generall Recognised As Safe
GTTAC	Gene Technology Technical Advisory Committee
ha	hectare
IgE	immunoglobulin E
<i>lec</i>	lectin gene
m	metre
mg/kg	milligram per kilogram
mm	millimetre
mRNA	messenger ribonucleic acid
NLRD	Notifiable Low Risk Dealing
<i>nos</i>	nopaline synthase gene
<i>npt</i>	neomycin phosphotransferase gene
OGTR	Office of the Gene Technology Regulator
PCR	polymerase chain reaction
RARMP	Risk Assessment and Risk Management Plan
RM	Risk Management
T-DNA	transfer deoxyribonucleic acid
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration
<i>vir</i>	virulence gene
WHO	World Health Organisation

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## EXECUTIVE SUMMARY

### THE REGULATION OF GENETICALLY MODIFIED ORGANISMS

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 and the environment that can not be managed. To this end, Section 51 of the Act requires the Regulator to prepare a risk assessment and risk management plan (RARMP) for each licence application, in consultation with a wide range of expert groups and stakeholders.

Under Section 52 of the Act, the Regulator is required to seek comment on the RARMP from those consulted in its preparation and to invite submissions from the public. Matters raised relating to the protection of human health and safety or the environment are taken into account in finalising the RARMP, which then forms the basis of the Regulator's decision on whether, or not, to issue a licence.

### THE APPLICATION

CSIRO Plant Industry (CSIRO) has applied for a licence (application number DIR 039/2003) for the limited and controlled release of genetically modified (GM) high oleic (HO) acid cotton into the environment. CSIRO proposes to conduct the trial for one growing season (2003 – 2004) at the Australian Cotton Research Institute (ACRI) in the Shire of Narrabri, NSW, covering a total of 2 hectares.

CSIRO proposes to release two GM cotton lines intended to increase the level of monounsaturated oleic acid (C18:1) and decrease the level of polyunsaturated linolenic acid (C18:2) in cottonseed. Both GM HO cotton lines were genetically modified with two genes, a modified fatty acid desaturase gene (*ghFAD2-1*) from cotton and an antibiotic resistance gene (neomycin phosphotransferase type II, *nptII*) from *Escherichia coli*. Short regulatory sequences control the expression of the introduced genes. The modified fatty acid enzyme is linked to the regulatory elements of a soybean lectin gene (*lec1*) to provide seed-specific gene function.

The modified fatty acid desaturase gene is expected to prevent the function of cotton's own desaturase enzyme and reduce conversion of oleic acid to linoleic acid. In glasshouse trials, the ratios of fatty acids in GM HO cottonseed are altered and contain higher oleic acid levels, and lower linoleic and palmitic acids, than non-GM cottonseed. No novel fatty acid is expected to be produced by GM HO cotton. The *nptII* gene confers resistance to the antibiotics kanamycin and neomycin. This gene was used in the early laboratory stages to select plant cells containing the desired genetic modification.

CSIRO's stated aims for the proposed field trials are:

- to conduct agronomic evaluation of GM HO cotton under field conditions; and
- to store seed from the release for testing the maintenance of high oleic acid levels in cottonseed.

No seeds will be retained for future plantings, as the applicant does not intend to conduct any future trials with these lines. If the proof of concept work is successful the novel trait will be bred into non-GM cotton varieties that are more suitable for commercialisation (subject to further approvals).

After the lint, cottonseed oil is the most valuable product derived from cotton plants. Oil from cottonseed is widely used in food applications around the world, following processing to remove gossypol and other toxic or anti-nutritional compounds such as cyclopropenoid fatty acids. The high levels of polyunsaturated fatty acids present in non-GM cottonseed oil often necessitates additional processing through partial hydrogenation to obtain oil with higher stability and more resistance to oxidation (ie to avoid becoming rancid). However, hydrogenation results in fatty acid structural forms (*trans*, rather than the *cis* arrangement of hydrogen atoms more commonly found in nature) that may increase cholesterol levels upon consumption.

GM HO cottonseed has an altered ratio of fatty acids, with increased oleic acid levels (monounsaturated fatty acid) and decreased levels of linoleic (polyunsaturated fatty acid with low stability) and palmitic acids (saturated fatty acid associated with blood cholesterol-raising properties). Oil from GM HO cottonseed is expected to have a greater stability than non-GM cottonseed oils. This may enable direct use in frying or for margarine hard stock, without the need for hydrogenation that current cottonseed oil requires.

Seed from the non-GM pollen trap rows will be destroyed and none of the cotton plants, or their by-products, will be used for animal feed or human food. Use of oil from GM HO cottonseed for human consumption will not be permitted as this would require prior approval by Food Standards Australia New Zealand (FSANZ). However, the CSIRO proposes to sell lint from the release. Lint does not contain genetic material, protein or fatty acids. Transport of the GM material, post-harvest management of the trial site and monitoring will be in accordance with guidelines issued by the Regulator.

GM HO cotton has not previously been approved for release in Australia. However, the use of the antibiotic selectable marker gene, *nptII*, has been thoroughly assessed in previous applications for field trials and general releases of GM cotton in Australia (refer to DIR 005/2001, DIR 006/2001, DIR 009/2002 and DIR 012/2002). In these applications, the introduction of the *nptII* gene into cotton was considered not to pose a significant risk to human health and safety, or the environment.

GM soybean with high oleic acid levels has been approved for commercial release in other countries. FSANZ has recently approved oil from GM HO soybean for human consumption. The applicant has not as yet applied to FSANZ for approval of GM HO cottonseed oil due to the early stage of the research.

## **THE EVALUATION PROCESS**

Licence application DIR 039/2003 from CSIRO has been evaluated and a RARMP prepared in accordance with the Act and the Regulations, using a Risk Analysis Framework. This framework was developed by the Regulator in consultation with the public and key State, Territory and Australian Government stakeholders and the Gene Technology Technical Advisory Committee, and is available at [www.ogtr.gov.au/pdf/public/raffinal.pdf](http://www.ogtr.gov.au/pdf/public/raffinal.pdf).

Details of the process that the Regulator must follow, including the prescribed consultation process on the application, and the matters that must be considered in preparing a RARMP, are

set out in Appendix 7 of the RARMP. The complete RARMP can be obtained from the OGTR or from the OGTR's web site at [www.ogtr.gov.au](http://www.ogtr.gov.au).

The risk assessment considered information relevant to the evaluation of potential impacts on human health and safety and the environment contained in the application (including information required by Act and the Regulations on the GMO, the parent organism, the proposed dealings and containment measures), submissions received during consultation with expert groups and authorities, and current scientific knowledge.

Through this process, potential hazards for human health and safety or the environment that may be posed by the proposed release of GM HO cotton were identified. These have been evaluated on the basis of the likelihood of each hazard occurring and the likely impact of the hazard, were it realised. Potential hazards that could arise from the genetic modifications, introduced gene products or altered traits, include:

- **toxicity and allergenicity for humans** : could GM HO cotton be more toxic or allergenic than non-GM cotton?
- **toxicity for other organisms** : could GM HO cotton be more harmful to other organisms than non-GM cotton?
- **weediness**: could GM HO cotton become a significant weed compared to non-GM cotton? and
- **transfer of introduced genes to other organisms** : could there be adverse consequences from potential transfer of the introduced genes to non-GM cotton crops, feral or native cottons, or to other organisms?

## CONCLUSIONS OF THE RISK ASSESSMENT

The Regulator considers that the limited and controlled release of GM HO cotton will not pose any significant risk to public health and safety, or to the Australian environment, that cannot be managed. The assessment of each potential hazard identified above is summarised under a separate heading below.

### **Toxicity or allergenicity to humans**

GM HO cotton is unlikely to prove more toxic or allergenic to humans than non-GM cotton. Expression of the modified fatty acid desaturase gene (derived from cotton) results in altered fatty acid ratios in cottonseed but no novel fatty acid is expected to be produced. The types of major fatty acid components in GM HO cottonseed, and their proportions, are similar to olive oil and other widely available oils. However, FSANZ is responsible for human food safety assessment, and FSANZ approval would be needed before products of these GM cottons could be used in human food.

Cottonseed from the proposed release will not be used for human food or animal feed. However, lint from the release will be sold commercially for use in fabric and other non-food products. Lint contains no DNA, protein or fatty acid.

The antibiotic protein, NPTII, is the same as that expressed in previously released GM cottons (DIR 005/2001, DIR 006/2001, DIR 009/2002 and DIR 012/2002). It is naturally widespread in the environment and has no known toxicity or allergenicity to humans.

## **Toxicity to other organisms**

GM HO cotton is unlikely to prove more toxic to other organisms than non-GM cotton and the limited scale of the trials would restrict the potential for exposure. Expression of the modified fatty acid desaturase gene (derived from cotton) results in altered fatty acid ratios in cottonseed but no novel fatty acids are expected to arise. The profile of the major fatty acid components in GM HO cottonseed is similar to olive oil and other widely available oils.

The antibiotic protein, NPTII, is the same as that expressed in previously released GM cottons (DIR 005/2001, DIR 006/2001, DIR 009/2002 and DIR 012/2002). It is naturally widespread in the environment and has no known toxicity to mammals, birds, fish, non-target invertebrates and soil microorganisms. Exposure of other organisms to the NPTII protein and GM cottonseed with its altered fatty acid composition will be low, and cottonseed from the release will not be used for stockfeed.

## **Weediness**

Cotton is not known to be a weed in Australia and has a low potential for dispersal by natural means. The modified traits in GM HO cotton (altered fatty acid ratios and antibiotic resistance) are unlikely to affect these characteristics. The major constraints on weediness of non-GM cotton, including soil moisture, nutrient availability, plant competition, herbivory, frost and fire are likely to apply equally to GM HO cotton. The antibiotic protein, NPTII, is the same as that expressed in previously released GM cottons (DIR 005/2001, DIR 006/2001, DIR 009/2002 and DIR 012/2002). It is not known to increase the potential for weediness of GM cotton.

## **Transfer of introduced genes to other organisms**

Although the overall frequency of out-crossing in cotton is very low, some gene transfer from GM HO cotton to other cultivated cottons would be likely under uncontrolled conditions. It is highly unlikely, however, that the inserted genes would increase the frequency of such gene transfers. Transfer of introduced genes to other cultivated cotton would pose the same risks as for GM HO cotton, which are assessed as low. Licence conditions have been imposed to minimise the transfer of introduced genes to other cotton crops (refer to key licence conditions below).

Transfer of introduced genes to feral/naturalised cotton is unlikely due to geographic isolation and the risk of transferring the introduced genes to native cottons is negligible because of hybrid infertility. The likelihood of transfer of the introduced genes to other organisms is negligible because of sexual incompatibility.

## **Additional data**

The proposed release is a proof of concept trial of these GMOs to test their abilities to produce cottonseed with high oleic acid when grown under field conditions. There is limited data on expression and molecular characterisation of the introduced genes. Information on altered plant properties is limited to glasshouse trials.

As genes inserted by genetic modification, can have an influence on multiple, sometimes unrelated, plant traits, unintended effects of the inserted genes may result in changes to characteristics that affect toxicity or allergenicity to humans, toxicity to other organisms, or weediness. The applicant proposes to assess the agronomic characteristics of GM HO cotton to identify any such unintended effects.

The applicant does not intend to conduct further trials with these lines. However, the evaluation process identified further data that, while not necessary for managing the risks posed by the proposed release, would be required before any future application for significantly larger scale trials with similarly modified lines or requests for reduced containment conditions could be evaluated.

### **THE RISK MANAGEMENT PLAN (KEY LICENCE CONDITIONS)**

As part of the evaluation process for this licence application, a risk management plan has been developed to address the risks identified (refer to Conclusion of the risk assessment, above). This plan is given effect by licence conditions. The key licence conditions are outlined below.

#### **Toxicity or allergenicity to humans**

Licence conditions have been imposed which require the applicant to:

- limit the scale of the release (single site of two hectares and one growing season);
- prevent entry of the GMOs and products derived from the GMOs into the human food supply;
- destroy all seed not required for assessment of the HO trait; and
- securely transport and store the GMOs.

#### **Toxicity to non-target organisms**

Licence conditions have been imposed which require the applicant to:

- limit the scale of the release (single site of two hectares and one growing season);
- prevent cottonseed from the trial being used as stockfeed;
- destroy all seed not required for assessment of the HO trait; and
- securely transport and store the GMOs.

#### **Weediness**

Licence conditions have been imposed which require the applicant to:

- limit the scale of the release (single site of two hectares and one growing season);
- surround the GM cotton lines by a 20 m pollen trap of non-GM cotton;
- securely transport and store the GMOs;
- prevent cottonseed from the trial being used as stockfeed;
- clean equipment used at the release site; and
- monitor release site after harvest and destroy volunteers.

#### **Transfer of introduced genes to other organisms**

Licence conditions have been imposed which require the applicant to:

- limit the scale of the release (single site of two hectares and one growing season);
- surround the GM cotton lines by a 20 m pollen trap of non-GM cotton;
- securely transport and store the GMOs;

- clean equipment used at the release site; and
- monitor release site after harvest and destroy volunteers.

In addition, the licence conditions require the applicant to undertake a research program to obtain data to validate previous research on the efficacy of the pollen trap.

### **General conditions**

The licence issued by the Regulator also contains a number of general conditions, which are also relevant to risk management. These include, for example,

- identification of the persons or classes of person covered by the licence;
- a requirement that the applicant allow access to the release site by the Regulator, or persons authorised by the Regulator, for the purposes of monitoring or auditing; and
- a requirement to inform the regulator if the applicant becomes aware of any additional information about risks to human health or safety or to the environment.

### **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. The OGTR also independently monitors releases that the Regulator has authorised. At least 20% of all field trial sites will be inspected each year, in accordance with a monitoring and compliance strategy based on risk profiling, to determine whether licence holders are complying with the licence conditions, or whether there are any unintended effects.

### **FURTHER INFORMATION**

Detailed information on the evaluation of the application, including the licence conditions, is available in the risk assessment and risk management plan document for this application, which can be obtained from the website of the Office of the Gene Technology Regulator ([www.ogtr.gov.au](http://www.ogtr.gov.au)), or by calling 1800 181 030 (please quote application number DIR 039/2003).

## CHAPTER 1 BACKGROUND

1. This chapter provides background information about the application and previous releases of relevant genetically modified organisms (GMOs) into the environment.
2. The OGTR has received an application (licence application number DIR 039/2003) from CSIRO Plant Industry for the intentional release into the environment of two genetically modified (GM) cotton lines containing a cotton gene to down regulate conversion of oleic fatty acid to linoleic fatty acid in the seed. The release is on a limited scale and under controlled conditions. Key information on the application is given below.

### SECTION 1 THE APPLICATION

Project Title	<b>Field evaluation of High-Oleic (HO) Cotton</b>
Applicant	CSIRO GPO Box 225 Dickson ACT 2602
Common name of the parent organism	Cotton
Scientific name of the parent organism	<i>Gossypium hirsutum</i> L.
Modified trait(s)	Altered fatty acid ratios in cottonseed and resistance to kanamycin related antibiotics
Identity of the gene(s) responsible for the modified trait(s)	<ul style="list-style-type: none"> <li>• <i>ghFAD2-1</i> gene from cotton (increased oleic acid, decreased linoleic and palmitic acids)</li> <li>• <i>nptII</i> gene from the bacterial Tn5 transposon (antibiotic resistance)</li> </ul>
Proposed Release Location	Shire of Narrabri, NSW
Proposed Release Size	One site of 2 hectares (about 15,000 plants)
Proposed Time of Release	Oct 2003 – May 2004

#### Section 1.1 The proposed dealings

3. CSIRO seeks approval for the limited and controlled release of high oleic GM cotton on one site of up to 2 hectares (about 15,000 plants) at the Australian Cotton Research Institute (ACRI) in the Shire of Narrabri, NSW. The trial is planned for the October 2003 – May 2004 cotton growing season.
4. The aims of the proposed release are to conduct agronomic evaluation of two GM cotton lines and to test for maintenance of the high oleic (HO) trait under field conditions. No seeds will be retained for future plantings, as the applicant does not intend to conduct any future trials with these lines. If the proof of concept work is successful the novel trait will be bred into non-GM cotton varieties that are more suitable for commercialisation (subject to further approvals).
5. None of the cotton plants from the release, or their by-products, will be used for animal and human food. However, the applicant proposes to sell lint from the release. Lint does not contain genetic material or protein.

## Section 1.2 Parent organism

6. The parent organism is cultivated cotton (*Gossypium hirsutum* L.), which is exotic to Australia and is grown as an agricultural crop in NSW and Qld and on a trial basis in WA and the NT. More detailed information on cotton can be found in a review document 'The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia' that was produced in order to inform the risk assessment processes for licence applications involving cotton. This document is available from the OGTR or at [www.ogtr.gov.au](http://www.ogtr.gov.au).

## Section 1.3 Genetic modification and its effect

7. Cotton plants were genetically modified with a construct that contains two genes, a modified form of a fatty acid desaturase (*FAD2-1*) from cotton (*gh*) and the neomycin phosphotransferase type II (*nptII*) selectable marker gene from *Escherichia coli*. The construct was inserted at different sites in two GM lines.

8. Short regulatory sequences control the expression of the introduced genes. The modified fatty acid desaturase gene is linked to the regulatory elements of a soybean lectin gene (*lec1*). The *nptII* gene is linked to the nopaline synthase regulatory elements from the soil bacterium *Agrobacterium tumefaciens*.

9. Expression of the modified fatty acid desaturase gene is expected to induce RNA based gene silencing, resulting in down regulation of cotton's own fatty acid desaturase gene and reduction in conversion of oleic acid to linoleic acid. In glasshouse trials, the ratios of fatty acids in GM HO cottonseed are altered and contain higher oleic acid levels, and lower levels of linoleic and palmitic acids, than non-GM cottonseed. No novel fatty acid is expected to be produced.

10. GM HO cotton contains the *nptII* gene that confers resistance to the related antibiotics kanamycin and neomycin. This gene was used as a selectable marker in the early laboratory stages to enable selection of plant cells containing the desired genetic modification.

11. Both GM HO cotton lines were genetically modified with the same gene cassette but have insertions at different sites in the genome.

12. Further details on the introduced genes and their effects are provided in Section 3 of Appendix 1. The evaluation of hazards relating to transfer of these genes to bacteria is discussed in Appendix 5.

## Section 1.4 Method of gene transfer

13. Two GM HO cotton lines were generated by introducing the gene construct into cotton at different sites on a plasmid vector carried by *Agrobacterium tumefaciens*. The vector is 'disarmed' as it lacks the genes that encode the tumour-inducing functions of *A. tumefaciens* (See Appendix 1, Section 4 for details).

14. Additional genetic elements derived from the plasmid vector can sometimes be transferred by *A. tumefaciens* to the plant. At present it is not known if any of these vector sequences are present in GM HO cotton.

## **SECTION 2 PREVIOUS RELEASES AND INTERNATIONAL APPROVALS**

### **Section 2.1 Previous Australian releases of similar GM cottons**

15. GM HO cotton has not been previously approved for release in Australia.

### **Section 2.2 Approvals by other Australian government agencies**

16. 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 GMOs, including the requirements of Food Standards Australia New Zealand (FSANZ).

17. Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment. FSANZ has approved oil from GM HO soybean (*Glycine max*) for human consumption. GM HO soybean was modified in a similar manner to GM HO cotton using the soybean endogenous *FAD2-1* gene to down regulate conversion of oleic acid to linoleic acid in soybean developing embryos. Consequently, GM HO soybean has a similar fatty acid profile to GM HO cotton with elevated levels of oleic acid and reduced levels of linoleic and palmitic acids.

18. Currently the applicant has not applied to FSANZ for evaluation of material from the GM HO cotton for use in human food. FSANZ approval would need to be obtained before it could be used in human food.

19. Further information about food safety and food labelling are available from FSANZ:

Food Standard 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](mailto:info@foodstandards.gov.au)  
<http://www.foodstandards.gov.au>

### **Section 2.3 International approvals for GM crops with high oleic fatty acid**

20. GM HO cotton has not been previously released in other countries. However, GM HO soybean was approved for commercial release in the USA and Puerto Rico (1997), in Japan (1999), and in Canada (2000). Additionally, GM HO soybean has been approved for both human food consumption and animal feed in Canada (2000), and in Japan (2000, 2001).

21. There have been no reports of adverse effects on human health or the environment resulting from the international release of GM HO soybean.

## **CHAPTER 2 SUMMARY OF RISK ASSESSMENT AND RISK MANAGEMENT PLAN**

22. The Act and the 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 and the environment (see Appendix 7).

### **SECTION 1 ISSUES RAISED IN SUBMISSIONS ON THE APPLICATION AND THE RISK ASSESSMENT AND THE RISK MANAGEMENT PLAN**

23. Comments received in response to consultation with expert groups and authorities on the preparation of the risk assessment and risk management plan (RARMP) under Section 50 of the Act, and with the same stakeholders and the public on the RARMP, under Section 52 of the Act, were very important in finalising the plan, which formed the basis of the Regulator's final decision on the application.

24. Written submissions on DIR 039/2003 raised the following issues relating to risks to human health and safety or the environment that have been addressed in the RARMP:

- the potential toxicity and allergenicity of GM HO cotton (Appendices 2 and 3);
- the potential for increased weediness of GM HO cotton (Appendix 4);
- the potential for, and management of, gene transfer to other cotton crops, naturalised cotton populations and native cottons (Appendix 5);
- the potential for adverse impacts arising from gene transfer to other organisms (Appendix 5);
- measures to limit the unintentional dispersal of GM cottonseed in the environment (Appendices 4, 5 and 6);
- unintended effects including gene silencing of non-target genes (Appendix 1); and
- additional data requirements for the future development of the GMOs (Chapter 2 and Appendix 6).

25. Prescribed agency submissions also raised issues such as impacts on existing and future export markets, ethical consequences of biotechnology, adverse effects on local cropping systems, economic and environmental viability of GM crops, and the potential deterioration of biodiversity, which are outside the scope of evaluations conducted under the Act and therefore have not been considered as a part of the assessment process.

26. In total the Regulator received two submissions from the public on this application. A summary of these written submissions is provided in Appendix 8. The key issues raised by the public that related to human health and safety or the environment were:

- potential for adverse impacts on human health (Appendix 2);
- potential for adverse impacts on the environment (Appendices 3, 4, and 5);
- management conditions to limit the spread of GMOs (Appendix 6); and
- conclusions regarding risks (Appendices 2-5).

27. Public submissions also raised issues such as impacts on domestic markets and export expansion, labelling of GM products, maintaining product integrity, food safety, and effective

tracking and tracing of GM material, which are outside the scope of evaluations conducted under the Act and therefore have not been considered as a part of the assessment process.

28. In accordance with Section 56 of the Act, the Regulator has taken into account all issues raised in written submissions that related to the protection of human health and safety and to the environment in preparing the risk assessment and the risk management plan. These issues were considered carefully and weighed against the body of current scientific information in reaching the conclusions set out in this document.

## **SECTION 2 PREPARATION OF THE RISK ASSESSMENT AND THE RISK MANAGEMENT PLAN**

29. The Regulator has conducted a risk assessment in relation to the proposed dealings and prepared a risk management plan. The risk assessment process, detailed in Appendix 7, identified a number of hazards that may be posed by the proposed dealings. The risks posed by these hazards were assessed as being either 'negligible', 'very low', 'low', 'moderate', 'high' or 'very high', by considering:

- the likelihood of the hazard occurring;
- the likely consequences (impact) of the hazard, were it realised; and
- risk management options to mitigate any identified risks.

30. The following table (Table 1) 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*. A comprehensive assessment of each identified hazard is provided in Appendices 2 - 5, as cross-referenced in the column headed *Summary of Risk Assessment*.

31. Where it is considered that risk management may be required to protect the health and safety of humans and/or the environment, the *Risk Management* column identifies the selected methods and the reasons they were chosen. A risk management plan for the proposed dealing has been given effect by specific conditions within the licence. These conditions are summarised in the final column, headed *Licence Conditions*, and detailed in Appendix 6.

**Table 1 Summary of the risk assessment and the risk management plan (including licence conditions)**

GM cotton: the genetically modified high oleic cotton proposed for release.  
 NPTII: neomycin phosphotransferase type II, confers resistance to the antibiotics neomycin and kanamycin.  
 N/A: not applicable.

Hazard Identification	Risk (combines 'likelihood' & 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reason(s) for selection	Is Risk Managed?	Licence conditions (See Appendix 6 for detailed licence conditions)
TOXICITY AND ALLERGENICITY FOR HUMANS: Food	Very Low	<p>See Appendix 2</p> <ul style="list-style-type: none"> <li>None of the GM material from the release will be used in human food;</li> <li>FSANZ approval would be required before the GM materials could be used for human food;</li> <li>expression of the introduced modified fatty acid desaturase results in altered fatty acid ratios in cottonseed but no novel fatty acids are expected to be produced;</li> <li>the proportion of oleic acid is increased to levels similar to olive oil;</li> <li>oleic acid is not known to be toxic or allergenic to humans; and</li> <li>the introduced NPTII protein is not toxic or allergenic.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><u>Prevent seed from entering human food supply</u>: prevents exposure through food.</li> <li><u>Destroy all seed not required for testing</u>: prevents unintended exposure.</li> <li><u>Ensure secure transport and storage of retained seed</u>: prevents unintended exposure.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><u>Prevent entry into human food supply</u>: no cottonseed to enter human food supply.</li> <li><u>Destroy seed</u>: destroy all seed not required for assessment of composition.</li> <li><u>Secure transport and storage</u>: the GMOs must not be transported unless contained within a primary, sealed container that is packed in a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within.</li> </ul>
TOXICITY AND ALLERGENICITY FOR HUMANS: Occupational exposure	Very Low	<p>See Appendix 2</p> <ul style="list-style-type: none"> <li>Cotton pollen is not wind-dispersed and therefore unlikely to be an air-borne allergen;</li> <li>expression of the introduced modified fatty acid desaturase results in altered fatty acid ratios in cottonseed but no novel fatty acids are expected to be produced;</li> <li>oleic acid is a component of all plant and animal cells;</li> <li>the introduced NPTII protein is already widespread in the environment;</li> <li>the introduced NPTII protein is not toxic or allergenic;</li> <li>there have been no reported toxic or allergic effects from similar GM cottons expressing the NPTII protein that have been extensively field trialed and are commercially released in Australia; and</li> <li>fibre characteristics of the GM cottons are likely to be the same as for non-GM cotton.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><u>Limit scale of release</u>: decreases likelihood of exposure.</li> <li><u>Destroy all seed not required for testing</u>: prevents unintended exposure.</li> <li><u>Ensure secure transport and storage of retained seed</u>: prevents unintended exposure.</li> <li><u>Report any adverse impacts on human health and safety</u>: ensures identification of unexpected adverse impacts.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><u>Limit scale</u>: restrict area to two hectares over one growing season.</li> <li><u>Destroy seed</u>: destroy all seed not required for assessment of composition.</li> <li><u>Secure transport and storage</u>: the GMOs must not be transported unless contained within a primary, sealed container that is packed in a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within.</li> <li><u>Report adverse impacts</u>: any adverse impacts on human health and safety must be reported to the Regulator.</li> </ul>
TOXICITY AND ALLERGENICITY FOR HUMANS: Wearing & using cotton products	Very Low	<p>See Appendix 2</p> <ul style="list-style-type: none"> <li>Cotton lint used in clothing and household items contains no proteins or DNA and cotton products from GM cottons can not be distinguished from non-GM products.</li> </ul>	No		N/A	None required

Hazard Identification	Risk (combines 'likelihood' and 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reason(s) for selection	Is Risk Managed?	Licence conditions (See Appendix 6 for detailed licence conditions)
<b>TOXICITY FOR OTHER ORGANISMS:</b> Mammals and wildlife including birds and fish	Very Low	See Appendix 3 <ul style="list-style-type: none"> <li>▪ None of the GM cotton material from the release will be used for animal feed;</li> <li>▪ expression of the introduced modified fatty acid desaturase results in altered fatty acid ratios in cottonseed but no novel fatty acids are expected to arise;</li> <li>▪ oleic acid is not known to be toxic to animals;</li> <li>▪ oleic acid is a component of all plant and animal cells;</li> <li>▪ the introduced NPTII protein is already widespread in the environment; and</li> <li>▪ the NPTII protein is not known to be toxic to any organism.</li> </ul>	Yes	<ul style="list-style-type: none"> <li>▪ <u>Limit scale of release</u>: decreases likelihood of exposure.</li> <li>▪ <u>Prevent seed from being used as stockfeed</u>: prevents exposure of animals.</li> <li>▪ <u>Destroy all seed not required for testing</u>: prevents unintended exposure.</li> <li>▪ <u>Ensure secure transport and storage of retained seed</u>: prevents unintended exposure.</li> </ul>	Yes	<ul style="list-style-type: none"> <li>▪ <u>Limit scale</u>: restrict area to two hectares over one growing season.</li> <li>▪ <u>Prevent seed from being used as stockfeed</u>: no cottonseed to be used as stockfeed.</li> <li>▪ <u>Destroy seed</u>: destroy all seed not required for assessment of composition.</li> <li>▪ <u>Secure transport and storage</u>: the GMOs must not be transported unless contained within a primary, sealed container that is packed in a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within.</li> </ul>
<b>TOXICITY FOR OTHER ORGANISMS:</b> Invertebrates, including soil insects	Very Low	See Appendix 3 <ul style="list-style-type: none"> <li>▪ Expression of the introduced modified fatty acid desaturase results in altered fatty acid ratios in cottonseed but no novel fatty acids are expected to arise;</li> <li>▪ oleic acid is not known to be toxic to invertebrates;</li> <li>▪ oleic acid is a component of all plant and animal cells;</li> <li>▪ the introduced NPTII protein is already widespread in the environment; and</li> <li>▪ the NPTII protein is not known to be toxic to any organism.</li> </ul>	No		N/A	None required
<b>TOXICITY FOR OTHER ORGANISMS:</b> Microbial organisms	Very Low	See Appendix 3 <ul style="list-style-type: none"> <li>▪ Expression of the introduced modified fatty acid desaturase results in altered fatty acid ratios in cottonseed but no novel fatty acids are expected to arise;</li> <li>▪ oleic acid is already widespread in the environment; and</li> <li>▪ the introduced NPTII protein is already widespread in the environment.</li> </ul>	No		N/A	None required.

Hazard Identification	Risk (combines 'likelihood' and 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reason(s) for selection	Is Risk Managed?	Licence conditions (See Appendix 6 for detailed licence conditions)
WEEDINESS:	Low	<p>See Appendix 4</p> <ul style="list-style-type: none"> <li>▪ The major constraints on weediness of non-GM cotton, such as water availability, nutrient availability, plant competition, herbivory, frost and fire, apply equally to GM HO cotton;</li> <li>▪ the introduced genes in GM HO cotton are unlikely to affect these characteristics;</li> <li>▪ the low potential for dispersal of cotton by natural means applies equally to GM HO cotton; and</li> <li>▪ there is a low possibility for the genetic modification to have potential pleiotropic effects that could alter traits affecting weediness, therefore, the applicant proposes to collect data on agronomic characteristics associated with weediness during the field trial.</li> </ul>	Yes	<ul style="list-style-type: none"> <li>▪ <u>Limit scale of the release</u> decreases likelihood of escape.</li> <li>▪ <u>Surround the GM cotton with a pollen trap</u>: minimises spread of the introduced genes beyond the release site via pollen flow.</li> <li>▪ <u>Ensure secure transport and storage of retained seed</u>: prevents escape of GM plant material outside the release site.</li> <li>▪ <u>Clean equipment used at the release site</u>: prevents escape of GM plant material into the environment outside the release site.</li> <li>▪ <u>Destroy any volunteers</u>: prevents persistence.</li> </ul>	Yes	<ul style="list-style-type: none"> <li>▪ <u>Limit scale</u>: restrict area to two hectares over one growing season.</li> <li>▪ <u>Surround the GM cotton with a pollen trap</u>: non-GM cotton must be grown on an area of land extending at least 20 m in all directions from the outside of the release site.</li> <li>▪ <u>Secure transport and storage</u>: the GMOs must not be transported unless contained within a primary, sealed container that is packed in a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within.</li> <li>▪ <u>Clean equipment used at the release site</u>: equipment must be cleaned before it is used for any other purpose. If GM cotton is ginned, the gin must be cleaned immediately following its use, before any other cotton is ginned.</li> <li>▪ <u>Destroy volunteers</u>: the release site must be monitored after harvest at least once every two months for at least 12 months and any cotton volunteers destroyed before flowering.</li> </ul>
<p>GENE TRANSFER:</p> <p>Plants</p> <ul style="list-style-type: none"> <li>• Other cotton crops</li> </ul>	Low	<p>See Appendix 5</p> <ul style="list-style-type: none"> <li>▪ Cotton is primarily self-pollinated;</li> <li>▪ gene transfer would pose the same risks as the low risks posed by GM HO cotton; and</li> <li>▪ gene transfer by pollen spread is very unlikely to spread beyond the proposed pollen trap.</li> </ul>	Yes	<ul style="list-style-type: none"> <li>▪ <u>Limit scale of the release</u> decreases potential transfer.</li> <li>▪ <u>Surround the GM cotton with a pollen trap</u>: minimises spread of the introduced genes beyond the release site via pollen flow.</li> <li>▪ <u>Ensure secure transport and storage of retained seed</u>: prevents escape of GM plant material outside the release site.</li> <li>▪ <u>Clean equipment used at the release site</u>: prevents escape of GM plant material into the environment outside the release site.</li> <li>▪ <u>Destroy any volunteers</u>: prevents persistence.</li> <li>▪ <u>Require applicant to develop a research program</u>: to monitor gene flow to other cottons.</li> </ul>	Yes	<ul style="list-style-type: none"> <li>▪ <u>Limit scale</u>: restrict area to two hectares over one growing season.</li> <li>▪ <u>Surround the GM cotton with a pollen trap</u>: non-GM cotton must be grown on an area of land extending at least 20 m in all directions from the outside of the release site.</li> <li>▪ <u>Secure transport and storage</u>: the GMOs must not be transported unless contained within a primary, sealed container that is packed in a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within.</li> <li>▪ <u>Clean equipment used at the release site</u>: equipment must be cleaned before it is used for any other purpose. If GM cotton is ginned, the gin must be cleaned immediately following its use, before any other cotton is ginned.</li> <li>▪ <u>Destroy volunteers</u>: the release site must be monitored after harvest at least once every two months for at least 12 months and any cotton volunteers destroyed before flowering.</li> <li>▪ <u>Applicant to develop a research program in consultation with the OGTR</u>.</li> </ul>

Hazard Identification	Risk (combines 'likelihood' and 'impact')	Summary of Risk Assessment (refer to appendices for details)	Does Risk Require Management?	Risk Management Method(s) and Reason(s) for selection	Is Risk Managed?	Licence conditions (See Appendix 6 for detailed licence conditions)
<b>GENE TRANSFER:</b> Plants <ul style="list-style-type: none"> <li>Feral naturalised cotton</li> </ul>	Low	<b>See Appendix 5</b> <ul style="list-style-type: none"> <li>Cotton is primarily self-pollinated;</li> <li>gene transfer would pose the same risks as the low risks posed by GM HO cotton; and</li> <li>geographical isolation of the trial site from populations of naturalised (feral) cottons.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><u>Limit scale of the release</u> decreases exposure.</li> <li><u>Surround the GM cotton with a pollen trap</u>: minimises spread of the introduced genes beyond the release site via pollen flow.</li> <li><u>Ensure secure transport and storage of retained seed</u>: prevents escape of GM plant material outside the release site.</li> <li><u>Clean equipment used at the release site</u>: prevents escape of GM plant material into the environment outside the release site.</li> <li><u>Destroy any volunteers</u>: prevents persistence.</li> <li><u>Require applicant to develop a research program</u>: to monitor gene flow to other cottons.</li> </ul>	Yes	<ul style="list-style-type: none"> <li><u>Limit scale</u>: restrict area to two hectares over one growing season.</li> <li><u>Surround the GM cotton with a pollen trap</u>: non-GM cotton must be grown on an area of land extending at least 20 m in all directions from the outside of the release site.</li> <li><u>Secure transport and storage</u>: the GMOs must not be transported unless contained within a primary, sealed container that is packed in a secondary unbreakable container; only transport to the extent necessary to store; store in sealed container within a locked facility that is signed to indicate GM cotton is stored within.</li> <li><u>Clean equipment used at the release site</u>: equipment must be cleaned before it is used for any other purpose. If GM cotton is ginned, the gin must be cleaned immediately following its use, before any other cotton is ginned.</li> <li><u>Destroy volunteers</u>: the release site must be monitored after harvest at least once every two months for at least 12 months and any cotton volunteers destroyed before flowering.</li> <li><u>Applicant to develop a research program in consultation with the OGTR</u>.</li> </ul>
<b>GENE TRANSFER:</b> Plants <ul style="list-style-type: none"> <li>Native cottons</li> </ul>	Negligible	<b>See Appendix 5</b> <ul style="list-style-type: none"> <li>Sexual incompatibility; and</li> <li>geographical isolation of the trial site from populations of native cottons.</li> </ul>	No		N/A	None required
<b>GENE TRANSFER:</b> Other plants	Negligible	<b>See Appendix 5</b> <ul style="list-style-type: none"> <li>Sexual incompatibility.</li> </ul>	No		N/A	None required
<b>GENE TRANSFER:</b> Humans & other animals	Negligible	<b>See Appendix 5</b> <ul style="list-style-type: none"> <li>None of the GM cotton material from the release will be used for human food or animal feed;</li> <li>FSANZ approval would be required before the GM materials could be used for human food;</li> <li>negligible opportunity for exposure of introduced genes to germ-line; and</li> <li>negligible occurrence of stable incorporation into the genome.</li> </ul>	No		N/A	None required
<b>GENE TRANSFER:</b> Microorganisms (including bacteria and viruses)	Negligible	<b>See Appendix 5</b> <ul style="list-style-type: none"> <li>Negligible persistence of rare transfer events due to strong negative selection pressures; and</li> <li>both of the introduced genes in GM HO cotton are already widespread in the environment.</li> </ul>	No		N/A	None required

### SECTION 3 DECISION ON THE APPLICATION

32. Details of the matters that the Regulator must consider in making a decision are provided in Appendix 7. 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.

33. It is concluded that there are no significant risks to human health and safety or to the Australian environment arising from the proposed release of GM high oleic cotton that cannot be adequately managed. Detailed risk analyses based on the available scientific information are provided in Appendices 2 - 5 in support of this conclusion.

34. Therefore the Regulator has issued licence number DIR 039/2003 in respect of this application.

### SECTION 4 RESEARCH REQUIREMENTS

35. As part of the OGTR's ongoing commitment to review of data, research conditions have been imposed for a number of cotton DIR licences. This research is intended to confirm previous research on pollen and gene flow undertaken prior to the implementation of the *Gene Technology Act 2000*. For the proposed release, research is required on the efficacy of pollen traps in controlling gene transfer through pollen to non-GM cotton and feral cotton.

36. Before any application for a significantly larger-scale release and/or reduced containment or commercial release of the GM HO cotton could be evaluated, more detailed information will be required:

- genetic segregation and molecular characterisation of the introduced genetic material;
- stability of the modified trait under Australian field conditions;
- altered composition of GM cottonseed under Australian field conditions;
- potential weediness of the GM cottons, including seed germination/dormancy characteristics;
- level of expression of NPTII protein; and
- unintended effects of the genetic modification.

37. It should be noted that provision of the above data during the proposed release are not required to ensure the management of risks to human health and safety and the environment from the proposed release. The risk management measures summarised in the Table above and given effect by the licence conditions, will achieve this purpose.

## APPENDIX 1 INFORMATION ABOUT THE GMO

38. 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 the genetic modification.

39. This part of the document addresses these matters and provides detailed information about the GMO proposed for release, the parent organism, the genetic modification process, the genes that have been introduced and the new proteins that are expressed in the genetically modified cotton.

### SECTION 1 SUMMARY INFORMATION ABOUT THE GMO

40. CSIRO proposes to release two lines of GM HO cotton under application DIR 039/2003 intended to down regulate conversion of monounsaturated oleic acid (C18:1) to polyunsaturated linolenic acid (C18:2) in cottonseed.

41. Cotton plants (*Gossypium hirsutum*) were genetically modified with a cassette that contains two genes, a modified form of a fatty acid desaturase (*FAD2-1*) from cotton (*gh*) and the neomycin phosphotransferase type II (*nptII*) selectable marker gene from *Escherichia coli*.

42. Short regulatory sequences (promoters and terminators) control the expression of the introduced genes. The modified *ghFAD2-1* gene is linked to the seed-specific regulatory elements of a soybean lectin gene (*lec1*). The *nptII* gene is linked to the nopaline synthase regulatory elements from the soil bacterium *Agrobacterium tumefaciens*.

43. Expression of the modified *ghFAD2-1* gene is expected to induce RNA based gene silencing, resulting in down regulation of the endogenous *ghFAD2-1* gene. Accordingly, there appears to be a reduction in the conversion of oleic acid to linoleic acid, resulting in elevated oleic acid levels in cottonseed from glasshouse trials.

44. GM HO cotton contains the *nptII* gene that confers resistance to the antibiotics kanamycin and neomycin. This gene was used as a selectable marker in the early laboratory stages to enable selection of plant cells containing the desired genetic modification. Potential hazards relating to transfer of these genes to bacteria are discussed in Appendix 5.

45. Further details on the introduced genes, their products and mechanism of action are provided in Section 3 of this Appendix.

### SECTION 2 THE PARENT ORGANISM

46. A comprehensive review of the parent organism, *Gossypium hirsutum* L. (cultivated cotton), is provided in the document, 'The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia' (OGTR 2002), available at [www.ogtr.gov.au](http://www.ogtr.gov.au).

47. The type of cotton cultivar used was Coker 315. This cultivar has been adapted to tissue culture conditions and can be routinely modified by standard genetic technologies. However, generation of agriculturally useful varieties requires multiple backcrosses of Coker 315 with elite cotton lines in order to transfer any useful trait into a preferred genetic background.

## SECTION 3 THE INTRODUCED GENES AND THEIR PRODUCTS

### Section 3.1 The modified fatty acid desaturase (high oleic) gene

48. A modified form of *ghFAD2-1* was introduced into cotton. In plants, fatty acid desaturase type 2 catalyses the conversion of the oleate (C18:1,  $\Delta^9$ <sup>cis</sup>) of phosphatidylcholine to the linoleate (C18:2,  $\Delta^9$ <sup>cis</sup>,  $\Delta^{12}$ <sup>cis</sup>) by introducing a double bond at the  $\Delta^{12}$  position, in the cis configuration. This enzyme, also described as oleoyl-phosphatidylcholine  $\Delta^6$ -desaturase or 1-acyl-2-oleoyl-sn-glycero-3-phosphocholine  $\Delta^{12}$ -desaturase, will be referred to as  $\Delta^{12}$ -fatty acid desaturase in this document.

49.  $\Delta^{12}$ -Fatty acid desaturase is a membrane-bound enzyme that is active in microsomal preparations from developing seed cotyledons of some species with oil rich seeds and is associated with the biosynthesis of triacylglycerols, which are composed of three fatty acid chains linked to a unit of glycerol. Activity of the enzyme is oxygen dependent as indicated by sensitivity to cyanide treatment and appears to require cytochrome *b*<sub>5</sub> and NADH: cytochrome *b*<sub>5</sub> reductase for activity (Smith et al. 1990).

50. The gene for  $\Delta^{12}$  fatty acid desaturase belongs to a small multigenic family in cotton, including *FAD2-1* and *FAD2-2*. Other members of the *FAD2* gene family may also be present in the cotton genome (Liu et al. 1999). In addition, cultivated cotton, *G. hirsutum*, is an allotetraploid species that has two copies of *FAD2-1*. The two copies of *ghFAD2-1* share about 98% nucleotide sequence identity. They contribute about equally to the conversion of oleic acid to linoleic acid in cottonseed. In contrast, diploid species of *Gossypium* have only one copy of *FAD2-1* (Liu et al. 1999).

Different forms of  $\Delta^{12}$  fatty acid desaturase are expressed in different tissues at different levels in cotton. The major  $\Delta^{12}$ -fatty acid desaturase activity in developing cottonseed embryos is due to *ghFAD2-1* enzyme. Expression of  $\Delta^{12}$ -fatty acid desaturase is developmentally regulated in cottonseed, peaking at 30-36 days after anthesis. No RNA transcripts of *ghFAD2-1* are detectable in leaves. In contrast, *ghFAD2-2* shows low level constitutive production of RNA transcripts throughout the plant, including seeds. The sequences of *ghFAD2-1* and *ghFAD2-2* are significantly divergent; they share only about 70% nucleotide sequence identity.

51. The *ghFAD2-1* gene was isolated from cotton (*G. hirsutum*) in the form of an 1,351 base pair fragment that includes the complete coding sequence, complete 3' non-coding sequence and partial 5' non-coding sequence. The modified form of the *ghFAD2-1* gene was constructed by duplicating an 853 base pair fragment from the 5' end of the gene and joined, in the opposite orientation, to the 3' end of the full-length version of the *ghFAD2-1* gene to create a 1½ inverted duplicate of *ghFAD2-1*.

52. The modified *ghFAD2-1* construct was linked to a promoter derived from a soybean (*Glycine max*) lectin gene (*lec1*). A promoter is a region of DNA that determines in which plant tissues a gene is expressed, when and to what extent it is expressed. The soybean lectin promoter has been tested with the  $\beta$ -glucuronidase reporter gene in GM cotton. Strong promoter activity was detected during embryo development, peaking late in embryo development (Townsend and Llewellyn, 2002). The *lec1* promoter activity appears to be seed specific in cotton and closely matches the expression pattern of endogenous *FAD2-1* gene activity.

53. Gene expression in plants is also regulated by the mRNA termination region, which includes a site for termination of RNA transcription and a polyadenylation signal. The 3' termination region can also influence stability of the mRNA and expression levels of protein.

The termination region for the modified *ghFAD2-1* gene was derived from the same soybean lectin gene used to provide the promoter region.

54. Potentially, the modified form of the *ghFAD2-1* gene could express a fully functional  $\Delta$ 12-fatty acid desaturase, which would be identical in structure and function to the endogenous non-GM cotton enzyme. However, Northern hybridisation data of the GM HO cotton line,  $\Delta$ 12-IR\*23, indicate that there is no detectable mRNA corresponding to either the modified form of *ghFAD2-1* or the endogenous *ghFAD2-1* gene. This is probably due to induction of gene silencing by RNA duplex formation of RNA transcripts of the inverted repeat region (Ahlquist, 2002), and thus  $\Delta$ 12-fatty acid desaturase activity is expected to be greatly reduced in GM HO cotton. More information on gene silencing is provided in Section 6 of this Appendix.

### **Section 3.2 Antibiotic resistance (neomycin/kanamycin) gene**

55. GM HO cotton contains the *nptII* antibiotic resistance marker gene from *E. coli*. This gene was used in the initial laboratory stages of development to enable selection of cells containing the desired genetic modification. The *nptII* gene is commonly used as a selectable marker in the production of GM plants.

56. The *nptII* gene was isolated from the bacterial Tn5 transposon (Beck et al. 1982). It encodes an enzyme, neomycin phosphotransferase type II (NPTII), which confers resistance to the aminoglycoside antibiotics kanamycin and neomycin. The NPTII enzyme uses ATP to phosphorylate kanamycin and neomycin, thereby inactivating the antibiotic and preventing it from killing the NPTII producing cell. The *nptII* gene functioned as a selectable marker in the initial tissue culture stage of cotton plant cell selection following genetic modification, allowing modified cells to grow while inhibiting the growth of non-modified cells.

57. The NPTII enzyme is widespread in the environment, in food chains, in naturally occurring kanamycin-resistant microorganisms found in soil, and in mammalian digestive systems (Flavell et al. 1992c).

58. Expression of the *nptII* gene in GM HO cotton plants is regulated by the promoter and mRNA termination region of the nopaline synthase gene (*nos*) from *A. tumefaciens*.

59. Potential hazards relating to the toxicity and allergenicity of the NPTII protein are discussed in Appendices 2 and 3, and those of antibiotic resistance gene transfer in Appendix 5.

### **Section 3.3 Other vector sequences**

60. In some instances gene transfer using *Agrobacterium tumefaciens* can result in the presence of additional vector sequences in the GM plant. The vector used by the applicant was derived from pBI121 (Liu et al. 2002), which was in turn derived from pBIN19 (Bevan 1984; Frisch et al. 1995) and contains the genetic elements *trfA*, *nptIII*, *is1*, *ori v* and *traF*. Incomplete or non-functional forms of *tetA*, *kilA* and the pUC *ori* also occur on this vector. These genetic elements were derived from *E. coli* and have been extensively used in molecular biology for more than 30 years without any evidence of adverse effects on human health. All of these elements usually function to support replication, maintenance, transfer or selection of DNA plasmids in *E. coli* or other compatible bacteria.

61. The presence or absence of any of these vector sequences in GM HO cotton has not been determined.

## SECTION 4 METHOD OF GENE TRANSFER

62. The GM HO cotton lines, ?12-IR\*23 and ?12-IR\*124 were produced by *Agrobacterium*-mediated transformation of the Coker 315 variety of cotton. *A. tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants. Plants can be genetically modified by the transfer of DNA (this segment of DNA is known as transfer-DNA or T-DNA and is located between specific border sequences on a resident plasmid) from *A. tumefaciens*, through the mediation of genes from the *vir* (virulence) region of Ti plasmids.

63. Disarmed *Agrobacterium* strains have been constructed specifically for plant transformation. The disarmed strains do not contain the genes (*iaaM*, *iaaH* and *ipt*) responsible for the overproduction of auxin and cytokinin, which are required for tumour induction and rapid callus growth (Klee & Rogers 1989). A useful feature of the Ti plasmid is the flexibility of the *vir* region to act in the *trans* configurations 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).

64. *Agrobacterium*-mediated transformation has been widely used in Australia and overseas for introducing new genes into plants without causing any reported biosafety problems.

65. In this instance, a conventional, disarmed, binary vector was used to introduce an 1½ inverted repeat of the *ghFAD2-1* gene into cotton variety Coker 315 using standard *Agrobacterium* transformation protocols. Genetically modified plants were selected for resistance to kanamycin in the laboratory.

66. Gene transfer using *A. tumefaciens* usually proceeds by generating a single-stranded DNA copy of the vector initiated at a nick in a specific sequence designated as the right border and terminating at the left border sequence (T-DNA). In some cases altered single-stranded DNA production patterns or recombination processes result in the incorporation into the plant genome of incomplete T-DNA segments or even additional vector sequences outside of the T-DNA. These additional vector sequences may be contiguous (linked) to the T-DNA or non-contiguous (unlinked) to the T-DNA.

67. The process of *Agrobacterium*-mediated plant transformation usually employs methods to control unwanted *Agrobacterium* growth. The most commonly applied method is the use of antibiotics such as cefataxime. However, this antibiotic arrests bacterial growth rather than killing unwanted bacterial cells. In addition, the bacterium may invade intercellular spaces of tissues, including seeds, if not exposed, or poorly exposed to the antibiotic. Consequently, some level of *Agrobacterium* persistence may occur.

68. There have been no reports of adverse consequences arising from the use of these disarmed forms of *Agrobacterium*.

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

69. DNA hybridisation of the *lec1* promoter to GM cotton total genomic DNA indicate that the introduced genetic elements are present as a single copy in both GM HO cotton lines, ?12-IR\*23 and ?12-IR\*124 (Liu et al. 2002). The Southern hybridisation data also indicate that the introduced DNA in each line has inserted at a different location of the cotton genome. The inheritance of HO as a dominant Mendelian trait with a segregation ratio of 3:1 is also compatible with a single copy transfer.

70. DNA hybridisation data, evidence of modified oleic and linoleic acid content (see Section 6), and resistance to the kanamycin antibiotic during selection suggest that all genetic elements in the T-DNA are present in both GM HO cotton lines.

71. Detailed information on the characterisation of the introduced genetic material would be required for any future application for a larger scale release of GM HO cotton.

## SECTION 6 EXPRESSION OF THE INTRODUCED GENES

72. In GM HO cotton, expression of the modified *ghFAD2-1* gene is intended to induce RNA based gene silencing (also known as post-transcriptional gene silencing, RNA interference, antisense, suppression, co-suppression or quelling). RNA based gene silencing results in the failure of messenger RNA to accumulate in the cytoplasm due to targeted degradation of the RNA. When the gene silencing system is triggered, the mRNA is degraded together with any RNA that has the same or closely similar sequence. Mechanistically, the main trigger for RNA based gene silencing is small double-stranded RNA segments of 21-25 base pairs, which provide sequence specificity and target degradation by double-stranded ribonucleases (Ahlgquist, 2002). Degradation of the mRNA therefore prevents production of the encoded protein.

73. The applicant expects that the *lec1* promoter derived from a soybean gene that encodes a seed lectin would drive seed-specific RNA transcription of the modified *ghFAD2-1* gene in GM HO cotton. This promoter is expected to achieve high levels of RNA transcription in cotton tissues where the endogenous *FAD2-1* gene is expressed, namely in developing embryos.

74. The production of RNA transcripts from the modified *ghFAD2-1* gene with its 1½ inverted repeat should result in the formation of double-stranded RNA, which could then trigger the RNA based gene silencing mechanism. It would be expected that RNA degradation should target not only the modified *ghFAD2-1* RNA transcripts but also RNA transcripts from both endogenous *ghFAD2-1* genes (98% nucleotide sequence identity). This would result in lack of *ghFAD2-1* protein product in cottonseed. However, the targeted RNA degradation may not be effective against the *ghFAD2-2* gene due to lower nucleotide sequence similarity (*ghFAD2-1* and *ghFAD2-2* share about 70% nucleotide sequence identity). Ineffective degradation of mRNA transcripts from *ghFAD2-2* would result in low levels of *ghFAD2-2* protein in leaves and seed.

75. Both GM HO cotton lines, ?12-IR\*23 and ?12-IR\*124, show altered fatty acid ratios in the oil fraction of cottonseed from glasshouse trials. GM HO cottonseed oil has elevated oleic acid content (about 75% of total fatty acids) compared to non-GM cotton (about 15%) and reduced linoleic acid content (about 6% of total fatty acids) compared to non-GM cotton (about 56%). Preliminary analysis of other fatty acids show little differences except for saturated palmitic acid (C16:0), which is reduced in GM HO cotton (<20%) compared to non-GM cotton (about 26%).

76. Although no protein expression data is available, Northern hybridisation data shows that ?12-IR\*23 has greatly reduced levels of *ghFAD2-1* RNA transcripts. The present data is consistent with sequence specific RNA based gene silencing of *ghFAD2-1* in cottonseed. The levels of *ghFAD2-2* do not appear to be affected as preliminary data shows that oleic acid content in leaves from GM HO cotton is equivalent to non-GM Coker 315 cotton leaves (information provided by the applicant).

77. A low level of linoleic acid is present in oil from both GM HO cotton lines, indicating some residual conversion of oleic acid to linoleic acid in cottonseed. This may be due to

inefficient gene silencing, non-overlapping spatial expression of RNA transcripts from the introduced gene and endogenous *ghFAD2-1*, or the result of other endogenous  $\Delta$ 12-desaturase activity that is not down-regulated due to a high degree of nucleotide sequence divergence relative to the modified *ghFAD2-1* sequence introduced into HO cotton. Other candidate  $\Delta$ 12-desaturases that function in cottonseed include FAD2-2 and FAD6. FAD6 acts on oleic acid bound to glycerolipid substrates in the plastid.

78. Expression of NPTII protein was required only during selection in tissue culture of genetically modified cotton cells following exposure to *A. tumefaciens*. Consequently, the stability of this altered trait has not been further examined. However, it is expected that the promoter derived from the nopaline synthase gene harboured by the Ti plasmid of native *A. tumefaciens* would allow constitutive expression of NPTII protein in all cotton progeny that carry the HO trait.

## **SECTION 7 STABILITY OF THE ALTERED TRAITS**

79. The stability of the HO trait has been established for four generations in the glasshouse. However, the maintenance of the HO trait under Australian field conditions is one of the major objectives of this field release. Three factors may influence the stability of the HO trait; i) environmental conditions, ii) stability of RNA induced gene silencing from generation to generation, and iii) infection by viruses in the field.

80. Gene silencing can be variable from cell to cell and generation to generation, resulting in mosaic patterns of disrupted gene expression (Napoli et al. 1990).

81. Plant viruses often contain genes that suppress host-induced gene silencing, which may result in reversion of the HO trait. Cotton plants are susceptible to at least 15 viruses, including Cucumber mosaic virus that is known to suppress gene silencing. In addition cotton can be infected by several *Ilaviruses* that are seed transmitted and could influence gene silencing of *ghFAD2-1* in subsequent generations of GM cotton. Although some viruses that infect cotton in the field may have little agronomic impact, infection may disrupt gene silencing of *ghFAD2-1* in the GM HO cotton lines. Glasshouse testing or monitoring virus presence in the field could provide information on the potential impact of viruses on stability of the HO trait.

82. If gene silencing fails to be triggered, then expression of  $\Delta$ 12-fatty acid desaturase in cottonseed could be restored or even increased compared to non-GM cotton. The modified *ghFAD2-1* gene contains one complete copy of the gene that encodes a functional enzyme. Expression of  $\Delta$ 12-fatty acid desaturase from both the introduced gene and the endogenous gene could increase the conversion of oleic to linoleic acid. Thus, lower proportions of oleic acid and higher proportions of linoleic acid could be produced in GM cottonseed, compared with results from glasshouse trials.

83. Detailed information on the expression of the introduced genes and stability of the altered traits would be required for any future application for a larger scale release of GM HO cotton.

## **SECTION 8 PLEIOTROPIC EFFECTS OF THE GENETIC MODIFICATION**

84. A single plant gene, including genes inserted by genetic modification, can have an influence on multiple, sometimes unrelated, plant traits. This phenomenon is known as pleiotropy. Therefore it is necessary to evaluate genetically modified plants for unintended, pleiotropic effects of the inserted genes, such as changes in agronomic characteristics.

85. Pleiotropic effects in GM HO cotton lines may arise from:

- disruption of gene function due to the site of insertion of the introduced genes into the cotton genome;
- secondary effects of altered oleic or linoleic acid content in GM HO cotton; and/or
- indirect effects on other parts of the same or different biochemical pathways.

### **Section 8.1 Unintended effects due to site of insertion**

86. DNA hybridisation data indicate that both GM HO cotton lines have a single copy of the introduced genes. No information is available on the site of insertion in the cotton genome. However, no unintended effects have been observed in the glasshouse (information supplied by applicant). In addition, the applicant plans to conduct evaluation of agronomic and phenotypic characteristics of the GM HO cotton as one of the major aims of the proposed field release.

### **Section 8.2 Secondary effects arising from down-regulation of the *ghFAD2-1* gene**

87. Down-regulation of expression from the cotton endogenous *ghFAD2-1* gene should reduce the conversion of oleic acid to linoleic acid in cottonseed. This is expected to lead to elevated oleic acid levels and reduced linoleic acid levels in cottonseed. Therefore, other biochemical pathways that make use of either oleic or linoleic acids, or their products, may be affected.

88. For example, oleic acid is a precursor to cyclopropanoid fatty acid synthesis. Preliminary analysis of fatty acid composition in GM HO cottonseed oil indicate that levels of two major cyclopropanoid fatty acids in cottonseed, malvalic and sterculic acids, are slightly elevated compared to that of non-GM cottonseed oil (information supplied by applicant). However, at values of less than 0.5% for both cyclopropanoid fatty acids, the levels are still within the range commonly found in different non-GM cotton varieties.

89. One possibility for the limited impact of oleic acid levels on cyclopropanoid fatty acid synthesis may be the distinct locations of different fatty acids in cottonseed. Oleic and linoleic fatty acids accumulate in the cotyledons of the developing embryo, whereas cyclopropane fatty acids are confined to the embryo axis (hypocotyl).

90. Decreased levels of linoleic acid (C18:2) in GM HO cotton does not appear to affect other processes. For example, conversion of linoleic to linolenic acid (C18:3) is low but the total level of linolenic acid (<1%) in the GM cotton is equivalent to that found in non-GM Coker 315 cotton (information supplied by the applicant).

### **Section 8.3 Other indirect effects**

91. The introduction of modified *ghFAD2-1* is intended to result in RNA based gene silencing of the endogenous gene. However, gene silencing may extend to other genes, in particular, other fatty acid desaturases in cotton, such as other members of the *ghFAD2* family. The likelihood of gene silencing corresponds to the degree of gene sequence relatedness and is unlikely to be effective against genes that share less than about 70-80% sequence identity with *ghFAD2-1*. The most closely related gene to *ghFAD2-1* that has been characterised in cotton is *ghFAD2-2*, which shares about 70% gene sequence identity with *ghFAD2-1*. Low levels of *ghFAD2-2* expression occur in leaves and seeds. Preliminary data supplied by the applicant on

the oleic acid levels in leaves and cottonseed indicates that the expression of *ghFAD2-2* may be unaffected.

92. From a preliminary analysis of the fatty acid composition of GM HO cottonseed oil, the levels of lauric (C12:0), myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), linolenic (C18:3), arachidic (C20:0) and arachidonic (C20:1) fatty acids are equivalent to non-GM Coker 315 cottonseed oil, with the exception of palmitic acid, which shows a significant decrease from about 26% to less than 20% of total fatty acid content in GM HO cottonseed oil. One possible explanation is that increased oleic acid levels in GM HO cottonseed results in increased levels of oleoyl-CoA in the cytoplasm, which may alter the selectivity of acyl-transferases responsible for the movement of the fatty acids into triglycerides in a way that selects against the incorporation of palmitate (Liu et al. 2002). A similar reduction in palmitic acid has also been observed in GM HO soybean.

93. Other possible indirect effects may include alteration of other cottonseed properties such as protein content, gossypol and other polyphenolic pigments (eg flavenoids), vitamins, minerals, or physiological properties such as altered dormancy or germination frequency. No information is available with regard to these possible indirect effects. Should future applications be made to continue work with the HO trait in Coker315 or cotton varieties that are more suitable for commercialisation then conditions could be imposed to gather this information to enable such applications to be assessed.

94. The applicant proposes to conduct evaluation of agronomic and phenotypic characteristics of GM HO cotton during this trial. This could include germination, growth habit, plant morphology, disease susceptibility, and seed and fibre characteristics such as length, strength, and diameter.

## APPENDIX 2 HUMAN HEALTH AND SAFETY

95. 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 risk management plan. This Appendix considers potential hazards that may be posed to human health and safety as a result of any toxicity or allergenicity of the GMOs, their novel proteins or novel traits.

### SECTION 1 NATURE OF THE POTENTIAL TOXICITY OR ALLERGENICITY HAZARD

96. Toxicity is the cascade of reactions resulting from exposure to a dose of chemical sufficient to cause direct cellular or tissue injury or otherwise inhibit normal physiological processes (Felsot 2000). Allergic responses are immune system reactions, resulting from stimulation of a specific group of antibodies (known as IgE) or sensitisation of specific tissue bound lymphocytes (FAO/WHO 2000; Taylor et al. 1996). Allergy has a well-defined biochemical cause that is different from toxicity.

97. HO cotton has been genetically modified by the introduction of two genes, a modified form of *ghFAD2-1* derived from cotton and the antibiotic resistance gene, *nptII*, derived from *E. coli* (see Appendix 1 for details of the genetic modification). Expression of the modified *ghFAD2-1* gene down regulates production of endogenous  $\Delta$ 12-fatty acid desaturase that converts oleic to linoleic acid in cottonseed. GM HO cotton has elevated levels of oleic acid and reduced levels of palmitic and linoleic acids in cottonseed. Only the ratios of fatty acids are altered, no novel fatty acid is expected to be produced by the genetic modification. Consequently, GM HO cotton differs from non-GM cotton in two traits, altered fatty acid ratios in cottonseed, and expression of the NPTII protein that confers resistance to the related antibiotics kanamycin and neomycin. The genetic modification may also have unintended effects due to either the site of insertion, secondary effects following expression of the introduced genes, or indirect effects, such as gene silencing of non-target genes.

98. The potential for GM HO cotton to be toxic or allergenic to humans due to either altered fatty acid composition in cottonseed, expression of the NPTII protein or because of unintended effects of the genetic modification is considered here.

### SECTION 2 LIKELIHOOD OF THE TOXICITY OR ALLERGENICITY HAZARD OCCURRING

99. In assessing the likelihood of adverse impacts due to toxicity or allergenicity of GM HO cotton on human health and safety, the following factors were considered:

- the inherent toxicity and allergenicity of non-GM cotton;
- the potential exposure to this GM cotton, its products, altered fatty acid composition and the new NPTII protein that is expressed in the cotton;
- the potential exposure to oleic acid and NPTII protein from other sources in the environment;
- the potential toxicity and allergenicity of the altered fatty acid composition and the NPTII protein expressed in the GM cotton; and
- any unintended effects that may affect the potential toxicity or allergenicity of GM HO cotton.

## Section 2.1 Toxicity and allergenicity of non-GM cotton

100. Information on the toxicity and allergenicity of non-GM cotton is included to establish a baseline for comparison with GM HO cotton. Attributes of non-GM cotton associated with potential toxicity and allergenicity to humans are discussed in the document 'The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia' (OGTR 2002) that was produced in order to inform the risk assessment processes for licence applications involving GM cotton. This document can be accessed at [www.ogtr.gov.au](http://www.ogtr.gov.au).

101. Cotton tissue, particularly the seeds, can be toxic if ingested in large quantities because of the presence of toxic and anti-nutritional factors including gossypol and cyclopropanoid fatty acids (eg. dihydrosterculic, sterculic and malvalic acids).

102. Cotton pollen is large, sticky and not transported easily by wind (OGTR 2002), therefore its potential to act as an airborne allergen is extremely low. However, inhalation of cotton dust by mill workers can cause byssinosis, an asthma-like condition, in sensitive individuals. Preventative measures such as the use of facemasks have been successful in lowering the incidence of this condition.

103. Cotton production is largely governed by demand for the fibre (lint and linters) surrounding the seed. The principle use of lint is in clothing, but other applications include medical supplies, yarns, felts, paper, plastics, food packaging and industrial fabrics. Processed cotton fibre contains 99.8% cellulose and is widely used in pharmaceutical and medical applications because of its very low allergenicity. Wearing and using cotton products is not known to be capable of causing disease or other ill health in people.

104. Although delinted cottonseed is a by-product of the cotton industry, it is used extensively in animal feed and as a source of oil for human consumption in the form of liquid oil, shortening, margarine and specialty products. Other minor uses of cottonseed include production of fertiliser, pharmaceuticals, soap, glycerine, fatty acids for industrial use, bran, fibre pulp, and as a source of furfural used in the manufacture of plastics and as a solvent in the refining of lubricating oils. In addition, cottonseed flour is a minor component in human food. In some countries cottonseed flour is a source of protein supplement. Cottonseed oil achieved GRAS (Generally Recognised As Safe) status under the United States Federal Food Drug and Cosmetic Act because of its common use prior to 1958 (ANZFA 2002).

105. The major components of cottonseed oil are edible fatty acids, including palmitic, oleic and linoleic acids (about 26%, 15% and 55% of total fatty acids, respectively). Minor amounts of myristic, palmitoleic, stearic and linolenic acids are also present. Fatty acids are necessary for cell membrane integrity, regulating cholesterol metabolism and infant brain development. In addition, some fatty acids are essential components in the diet due to the lack of specific fatty acid desaturases in humans and other mammals. For example, linoleic and linolenic (C18:3) acids serve as precursors of eicosanoids including prostaglandins, thromboxanes and leukotrienes, and are only available in through dietary intake.

## Section 2.2 Exposure of people to GM HO cotton

106. One of the aims of this controlled and limited release is to produce seed from the release to analyse the high oleic acid trait in oil extracted from GM cottonseed produced under field conditions. This would require storage and chemical analysis of the GM cottonseed in laboratory facilities approved by the Regulator. None of the GM material from the release will

be used in human food. Approval by FSANZ would be required before the GM materials could be used for human food.

107. Although none of the GM material from the release will be used as food, the genetic modification is intended to alter properties of a widely used human food product, namely, cottonseed oil. Therefore, the potential effects of the altered fatty acid ratios on human health and safety are considered here briefly, relative to non-GM cotton.

108. The applicant proposes to sell the lint produced in the release for use in fabric, upholstery and other non-food products. Cottonseed oil and cotton linters are highly refined and processed, with no detectable DNA (genetic material) or proteins (Leffler & Tubertini 1976; Sims et al. 1996).

109. Hence, exposure of people to GM HO cotton will be considered with respect to:

- cottonseed products in food, in particular, cottonseed oil;
- working with cotton or living in or near the areas where cotton is grown; and
- wearing cotton clothing or using household items made from cotton lint.

### **2.2.1 Exposure through food**

110. Although none of the GM material from the release will be used as food, the genetic modification is intended to alter properties of a widely used human food product, namely, cottonseed oil. Approval by FSANZ would be required before the GM materials could be used for human food.

111. Expression of the modified *ghFAD2-1* gene down regulates production of endogenous  $\Delta$ 12-fatty acid desaturase that converts oleic to linoleic acid in developing embryos in cottonseed, resulting in accumulation of oleic acid in GM HO cottonseed. The total amount of oil extracted from GM HO cottonseed is unchanged, only the ratios of fatty acids are altered. In particular, the proportion of oleic fatty acid is increased (about 15% ? 75%), with concomitant decreases in linoleic acid (about 56% ? 6%) and palmitic acid (about 26% ? 16%). These changes result in a fatty acid profile that is more closely similar to olive oil and high oleic varieties of canola, safflower and sunflower. No novel fatty acids are produced by the genetic modification.

112. Whereas humans can synthesise oleic acid from other fatty acid precursors, in particular stearic acid, we lack the appropriate desaturases for converting oleic to linoleic acid. Therefore, linoleic acid is considered an essential fatty acid in the diet. In addition, linoleic acid imparts desirable flavours in sensory tests. The commonly recommended minimum amount of dietary linoleic acid is about 1-2% of total energy. GM HO cottonseed oil retains a low level of linoleic acid (see Appendix 1, Section 6 for more details).

113. Vegetable oils with a high proportion of oleic acid relative to polyunsaturated fatty acids have high oxidative stability when used in frying, baking and other food processes. Consequently, HO oils have less need for partial hydrogenation when used for food industry applications. Reducing the levels of hydrogenation reduces the level of *trans* fatty acids consumed and thus is likely to reduce the development of unfavourable cholesterol profiles linked to cardiovascular disease. However, partial hydrogenation of cottonseed oil is also used to reduce the levels of antinutritional cyclopropene fatty acids.

114. The altered fatty acid composition of GM HO cotton does not change the types of fatty acids found in cottonseed, but only alters the ratios of palmitic, oleic and linoleic acids. None of these fatty acids is known to be toxic to humans.

115. The NPTII protein has been introduced into a number of other GM cottons that have been released into the Australian environment (see DIR licences 005/2001, 006/2001, 009/2002 and 012/2002). Thorough risk analysis of these DIR applications have concluded that GM cottons expressing the NPTII protein are unlikely to enhance toxicity to humans relative to non-GM cotton.

116. GM HO cottonseed has a similar edible fatty acid profile to GM HO soybean seed, which has been approved for commercial release in Canada and the USA. FSANZ has approved oil from GM HO soybean seed for human consumption.

### **2.2.2 Exposure to GM cotton through working with cotton or living near cotton crops**

117. Cotton is a well-established field crop with a long history of safe use. Cotton pollen is large, sticky and not transported easily by wind (OGTR 2002), thereby limiting exposure to cotton pollen as a potential airborne allergen for cotton workers or people living near cotton crops. However, processing of cotton at cotton gins, and the bulk handling of cottonseed and cotton fibre, can create and stir up fine dust and lint particles. Inhalation of cotton dust by mill workers can cause byssinosis, an asthma-like condition, in sensitive individuals. The use of facemasks is common practice in cotton factories and has been successful in lowering the incidence of this condition.

118. Humans working with cotton plants would be exposed primarily to the outer waxy cuticle layer at the plant surface, to the seed coat or to the cotton fibres, all of which are essentially free of protein and fatty acids.

119. The altered fatty acid composition of GM HO cotton does not change the types of fatty acids found in cottonseed, but only alters the ratios of palmitic, oleic and linoleic acids. None of these fatty acids is known to be toxic to humans. It is not expected that these altered fatty acid ratios will affect other properties of GM HO cotton that may impact on human health and safety when working with or living near GM HO cotton.

120. Exposure to the NPTII protein or to other cellular components of GM HO cotton plants will only occur if plant cells are ruptured. The NPTII protein is expected to be expressed at very low levels in the pollen of GM HO cotton, based on the similarity of the promoter elements and expression levels of NPTII to other GM cottons (see DIRs 005/2001, 006/2001, 009/2002 and 012/2002).

121. The fibre characteristics (length, strength, fineness) of GM HO cotton is expected to be equivalent to non-GM cotton. The cotton lint derived from GM HO cotton is no more likely to induce adverse responses in workers than is non-GM cotton. However, the applicant proposes to assess the fibre characteristics of GM HO cotton as part of the proposed trial.

122. Therefore, it is expected that the altered fatty acid composition and the introduced NPTII protein will not significantly enhance the toxicity or allergenicity of GM HO cotton for cotton workers or people living near cotton crops relative to non-GM cotton. More comprehensive examination of plant characteristics is proposed as part of the field evaluation to test for any unintended, pleiotropic effects.

### 2.2.3 Exposure to GM cotton products through wearing clothing and using household products made from cotton lint

123. Cotton fabrics, used in clothing, upholstery, towels and other household products, are made from the cotton lint (long fibres) which surrounds the cottonseed. Processed cotton fibre contains 99.8% cellulose and has no genetic material. Household products that may contain cotton linters (short fibres) include medical dressings, felt, fine quality paper (including banknotes in many countries), twine and mops. Cellulose derivatives produced from the linters may be used in pharmaceuticals, cosmetics, toothpaste, lacquers, paints and variety of plastics. Cotton linters are widely used in pharmaceutical and medical applications because of their very low allergenicity.

124. Processed cotton lint, linters and oil contain no detectable DNA or protein (Leffler & Tubertini 1976; Sims et al. 1996). Fibre characteristics (length, strength, fineness) of GM HO cotton are expected to be equivalent to non-GM varieties. However, the applicant proposes to assess the fibre characteristics of GM HO cotton as part of the proposed trial. GM HO cotton is expected to differ from non-GM cotton in the expression of the NPTII protein and in altered fatty acid ratios in cottonseed, neither trait is present in cotton lint. Therefore the safety of wearing cotton clothing or using other products made from GM HO cotton is not likely to be different from that of non-GM cotton.

### Section 2.3 Other sources of oleic acid and NPTII in the environment

125. Oleic, linoleic and palmitic acids are not known to be toxic or allergenic and are present in most edible oils. Oleic acid is an abundant component of all plant and animal cell membranes and fat storage structures. Edible fatty acids collectively are an essential component of the diet. Current recommended levels of dietary fatty acids is 15-30% of total energy intake. More specifically, it is recommended that <10% of total energy comes from saturated fatty acids, 6-10% polyunsaturated fatty acids, <1% *trans* fatty acids and the remaining proportion comes from monounsaturated fatty acids (WHO, 2003).

126. Nevertheless, the ratios of different fatty acids in the diet can alter the composition of membrane structures, the levels and composition of plasma lipids and the balance of essential fatty acid metabolites in serum. Alterations in plasma lipids and fatty acid metabolites can impact variably, not only on normal physiological functions such as immune responses or homeostatic functions (eg temperature and sleep), but also on a number of chronic diseases such cardiovascular disease and type 2 diabetes. In addition, a number of other chronic diseases are associated with abnormalities in fatty acid incorporation into cell membrane structures and circulating fatty acid metabolites, including lupus erythematosus, Kawasaki's disease, chronic granulomatous leukaemia, allergies, arterogenesis, thrombosis, arteriosclerosis, peripheral vascular disease, gout, psoriasis, atopic dermatitis, coeliac disease, chronic inflammatory disease and chronic fatigue syndrome. A number of these diseases may be influenced by the type of fatty acids that are consumed.

127. High levels of saturated fatty acids, such as palmitic and myristic acids, are convincingly linked to an increased risk of cardiovascular disease and type 2 diabetes. The reduced levels of palmitic acid in oil from GM HO cottonseed result in a lower ratio of saturated to unsaturated fatty acid. Potentially, this results in a more favourable fatty acid profile with respect to human health.

128. It has been demonstrated that diets with polyunsaturated fatty acids replacing saturated fatty acids reduce the risk of cardiovascular disease by lowering plasma levels of low density

lipoprotein-cholesterol (Kris-Etherton, 1999). While some studies suggest that polyunsaturated fatty acids (such as linoleic acid) may be more effective than monounsaturated fatty acids (such as oleic acid) in lowering low density lipoprotein-cholesterol (Howard et al. 1995; Kris-Etherton 1999), other studies suggest that both types of dietary fatty acid may be equally effective in fostering favourable plasma lipid profiles than saturated fatty acids (Navarro et al. 1992; Nydahl et al. 1994; Grundy 1997; Baroni et al. 1999; Trautwein et al. 1999; Hodson et al. 2001; Kratz et al. 2003).

129. The NPTII protein provides resistance to the related antibiotics kanamycin and neomycin, and is found in a number of bacteria that are widespread in the environment, including bacteria of the human gut. The NPTII protein can be found in or on fresh food. Humans continually ingest kanamycin-resistant microorganisms, some containing the NPTII protein. The diet, especially raw salad, is the major source of NPTII. At a conservative estimate, each human ingests  $1.2 \times 10^6$  kanamycin-resistant microorganisms daily (Flavell et al. 1992b). Large numbers of kanamycin or neomycin resistant bacteria already inhabit the human digestive system (Levy et al. 1998), with Flavell et al. (1992) estimating about  $10^{12}$  per person.

## **Section 2.4 Potential toxicity and allergenicity of the modified fatty acid ratios and NPTII protein**

130. The potential for enhanced toxicity or allergenicity of GM HO cotton compared to non-GM cotton, Coker 315, depends on the effects of the modified traits, including altered fatty acid ratios in cottonseed, expression of the antibiotic *nptII* gene and unintended pleiotropic effects. These modified traits may impact on human consumption of cottonseed products, exposure through working with cotton, or exposure to cotton in clothing or household items.

### **2.4.1 Toxicity**

#### OLEIC ACID

131. Only the ratios of fatty acids in cottonseed are altered, no novel fatty acid is expected to be produced by the introduced modified *ghFAD2-1* gene. The proportions of the major edible fatty acids in GM HO cottonseed oil are similar to olive oil. The proportion of oleic acid is increased GM HO cottonseed. Oleic acid is an abundant component of all plant and animal cell membranes and fat storage structures. Oleic acid has no known toxicity.

#### THE NPTII PROTEIN

132. An acute oral toxicity study in mice, in which the purified NPTII protein was fed at doses of up to 5000 mg/kg of body weight (2500 mg/kg administered twice, four hours apart), did not show any adverse effects (Berberich et al. 1993). The US FDA has concluded that NPTII does not possess any properties that would distinguish it toxicologically from other phosphorylating enzymes in the food supply, and which are present in all plants and animals. NPTII is approved as an additive in food for human consumption in the US (FDA 1994). The US EPA has also established an exemption for NPTII from the requirement for a residue tolerance limit when used as a plant pesticide inert ingredient (EPA 1994).

133. The NPTII protein has been introduced into a number of other GM cottons that have been released into the Australian environment (see DIR licences 005/2001, 006/2001, 009/2002 and 012/2002). Thorough risk analysis of these DIR applications have concluded that GM cottons expressing the NPTII protein are unlikely to enhance the risk to human health and safety relative

to non-GM cotton. Furthermore, GM HO cottonseed produced in the field trial will not be used in human food or animal feed.

## 2.4.2 Allergenicity

### OLEIC ACID

134. Fatty acids do not display characteristics common to known food allergens. Only the ratios of fatty acids in cottonseed are altered, no novel fatty acid is expected to be produced by the introduced modified *ghFAD2-1* gene. The proportions of the major edible fatty acids in GM HO cottonseed oil are similar to olive oil. The proportion of oleic acid is increased but has no known allergenic properties. Studies with GM HO soybeans with high oleic acid found no differences in allergen content from non-GM soybean (Lehrer & Reese, 1997).

### THE NPTII PROTEIN

135. Although there are no predictive assays available to assess the allergenic potential of proteins, much is known about the biochemical events associated with allergic reactions, as well as the kinds of proteins that cause problems (Metcalf et al. 1996; Taylor & Lehrer 1996). For example, food allergens are usually present as a major component of the ingested food and are resistant to heat, protease digestion and to the acid conditions of the stomach (Astwood et al. 1996; Metcalfe et al. 1996; Taylor & Lehrer 1996; Kimber et al. 1999). The allergenic proteins of many major sources of allergens, including food allergens, have been characterised by molecular means, allowing comparisons to be made as a useful step in assessment of allergenic potential (Metcalf et al. 1996).

136. The NPTII protein does not display characteristics common to known food allergen proteins (Fuchs et al. 1993b; FDA 1994; FDA 1998; ANZFA 2000; ANZFA 2001). The NPTII protein is heat labile and degrades rapidly in simulated human gastric fluid. Fuchs et al. (1993a) reported that no NPTII was detected 10 seconds after addition of simulated gastric fluid as measured by both Western blot analyses and enzymatic activity. NPTII shows no significant DNA or protein sequence homology to known allergens in the EMBL, Genbank, Pir and SwissProt protein databases (Fuchs & Astwood 1996).

## Section 2.5 Other unintended effects

137. A single plant gene, including genes inserted by genetic modification, can have an influence on multiple, sometimes unrelated, plant traits. This phenomenon is known as pleiotropy. Therefore it is necessary to evaluate genetically modified plants for unintended, pleiotropic effects of the inserted genes, such as changes in toxins or allergens.

138. Pleiotropic effects in GM HO cotton lines may arise from:

- disruption of gene function due to the site of insertion of the introduced genes into the cotton genome;
- secondary effects of altered oleic or linoleic acid content in GM HO cotton; and/or
- indirect effects on other parts of the same or different biochemical pathways.

139. Some of these unintended effects may alter traits that impact on human health and safety. Data from plants grown under glasshouse conditions indicate that both HO lines, ?12-IR\*23 and ?12-IR\*124, have normal appearance and growth vigour, with no detectable differences compared to non-GM cotton (information supplied by the applicant). However, effects of the

genetic modification on cottonseed composition, in particular, complete fatty acid profiles (including cyclopropenoids), protein content, and levels of gossypol, polyphenolic pigments, minerals and vitamins, have not been determined. A more comprehensive examination of plant characteristics is proposed as part of the field evaluation. (See Appendix 1, Section 8 for a more detailed consideration of pleiotropic effects.)

### **SECTION 3 CONCLUSIONS REGARDING TOXICITY OR ALLERGENICITY**

140. The proposed release is the first proof of concept trial of GM HO cotton to test its potential for producing cottonseed with high oleic acid when grown under Australian field conditions. There is limited data on expression and molecular characterisation of the introduced genes and information on altered plant properties is restricted to glasshouse trials. Nevertheless, it is considered that the risk of GM HO cotton being more toxic or allergenic for humans than non-GM cotton is very low because:

- none of the GM material from the release will be used in human food or animal feed;
- expression of the introduced modified fatty acid desaturase results in altered fatty acid ratios in cottonseed but no novel fatty acids are expected to be produced;
- the proportion of oleic acid is increased to levels similar to olive oil and high oleic canola, safflower and sunflower oils, which have well established records of safe use in food;
- oleic acid is not known to be toxic or allergenic to humans;
- oleic acid is a component of all plant and animal cells;
- the introduced NPTII protein is not toxic or allergenic;
- the introduced NPTII protein is already widespread in the environment;
- there have been no reported toxic or allergic effects from similar GM cottons expressing the NPTII protein that have been extensively field trialed and are commercially released in Australia;
- cotton pollen, whose properties are expected to be essentially the same in GM HO and non-GM cottons, is not wind-dispersed and therefore unlikely to be an air-borne allergen;
- fibre characteristics of the GM cottons are likely to be the same as for non-GM cotton, with no greater propensity for respiratory irritation through exposure to cotton dust or lint; and
- cotton lint used in clothing and household items contains no proteins or DNA and cotton products from GM cottons can not be distinguished from non-GM products.

141. FSANZ approval would be required before the GM materials could be used for human food.

142. As a condition of licence, CSIRO must report any adverse effects on human health and safety (for example allergic reactions as a result of occupational exposure to the cotton) or to the environment.

## APPENDIX 3 TOXICITY TO OTHER ORGANISMS

143. 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 risk management plan. This Appendix considers potential hazards, in particular toxicity, which may be posed to other organisms due to the genetic modification.

### SECTION 1 NATURE OF THE POTENTIAL TOXICITY HAZARD

144. HO cotton has been genetically modified by the introduction of two genes, a modified form of *ghFAD2-1* derived from cotton and the antibiotic resistance gene, *nptII*, derived from *E. coli* (see Appendix 1 for details of the genetic modification). Expression of the modified *ghFAD2-1* gene down regulates production of endogenous  $\Delta^12$ -fatty acid desaturase that converts oleic to linoleic acid in cottonseed. GM HO cotton has elevated levels of oleic acid and reduced levels of palmitic and linoleic acids in cottonseed. Only the ratios of fatty acids are altered, no novel fatty acid is expected to be produced by the genetic modification. Consequently, GM HO cotton differs from non-GM cotton in two traits, altered fatty acid ratios in cottonseed, and expression of the NPTII protein that confers resistance to the related antibiotics kanamycin and neomycin. The genetic modification may also have unintended effects due to either the site of insertion, secondary effects following expression of the introduced genes, or indirect effects, such as gene silencing of non-target genes.

145. In addition to humans, the modified traits may have adverse impacts on a wide range of other organisms in the environment that are exposed to GM HO cotton or its products. These adverse impacts may be due to the direct toxicity of the new traits on other organisms or indirectly through predators or parasitoids of organisms that feed or live on cotton. Effects of the altered traits may also be manifested in altered fitness of other organisms, resulting in adverse impacts on biodiversity.

146. The potential for GM HO cotton to be toxic to other organisms due to either altered fatty acid composition in cottonseed, expression of the NPTII protein or because of unintended effects of the genetic modification is considered here, including potential adverse impacts on the following groups of other organisms:

- wildlife, including mammals, fish and birds;
- invertebrates, including beneficial insects (pollinators, parasitoids or predators of insect pests); and
- microbial organisms, particularly soil microorganisms.

### SECTION 2 LIKELIHOOD OF THE TOXICITY HAZARD OCCURRING

147. In assessing the likelihood of adverse impacts due to toxicity or allergenicity of GM HO cotton on human health and safety, the following factors were considered:

- the inherent toxicity and allergenicity of non-GM cotton;
- the potential exposure to this GM cotton, its products, altered fatty acid composition and the new NPTII protein that is expressed in the cotton;
- the potential exposure to oleic acid and NPTII protein from other sources in the environment;

- the potential toxicity of the altered fatty acid composition and the NPTII protein expressed in the GM cotton; and
- any unintended effects that may affect the potential toxicity or allergenicity of GM HO cotton.

## **Section 2.1 Toxicity of non-GM cotton**

148. Information on the toxicity of non-GM cotton is included to establish a baseline for comparison with GM HO cotton. Attributes of non-GM cotton associated with potential toxicity to other organisms are discussed in the document ‘The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia’ (OGTR 2002) that was produced in order to inform the risk assessment processes for licence applications involving GM cotton. This document can be accessed at [www.ogtr.gov.au](http://www.ogtr.gov.au).

149. Cotton tissue, particularly the seeds, can be toxic if ingested in large quantities because of the presence of toxic and anti-nutritional factors including gossypol and cyclopropanoid fatty acids (eg. dihydrosterculic, sterculic and malvalic acids). Most mammals avoid feeding on cotton due to the presence of gossypol and other components of cotton tissues and the morphology of the plant (OGTR 2002). In the field, seed cotton is present as large lint-covered seeds that are unattractive to avian species (OGTR 2002). Current pesticide use restricts diversity of wildlife and, in particular, insects in cotton crops.

150. The presence of gossypol and cyclopropanoid fatty acids in cottonseed limits the use of whole cottonseed as a protein supplement in animal feed, except for cattle, which are less affected by these components. Inactivation or removal of these components during processing enables the use of some cottonseed meal for catfish, poultry and swine. The meal and hulls of cottonseed can also be used for cattle feed. Its use as stockfeed is limited, nonetheless, to a relatively small proportion of the diet and it must be introduced gradually, to avoid potential toxic effects.

151. Best Management Practices for the Australian cotton industry prohibits the use of cotton trash and stubble as a feed for animals, due to residues of pesticides that could be found in the cotton trash and stubble.

## **Section 2.2 Exposure of other organisms to GM HO cotton**

### **2.2.1 Livestock and wildlife**

152. None of the cotton plants from the release, or their by-products, will be used as stockfeed. GM HO cotton differs from non-GM cotton in two traits, altered fatty acid ratios in cottonseed and expression of the NPTII protein that confers resistance to the related antibiotics kanamycin and neomycin. The levels of cyclopropanoid fatty acids in GM HO cottonseed are equivalent to non-GM cottonseed and the GM HO plants have normal growth, appearance and vigour in glasshouse conditions (information supplied by the applicant). The level of gossypol is not expected to be altered. Toxins and anti-nutritive compounds in cotton leaves and seed deter consumption by most animals. The genetic modification of HO cotton does not appear to alter those properties.

153. Cottonseed or pollen is not expected to enter aquatic habitats in any significant quantity, limiting exposure of aquatic organisms. Irrigation practices (Good Management Practice of cotton industry) used by cotton growers in Australia retain irrigation water run-off, as well as the first 15 mm of storm water run-off, on farm to minimise the entrance of pesticide residues

into natural waterways. The licence includes a requirement to separate cotton trials from natural waterways by at least 50 m.

### **2.2.2 Invertebrates**

154. Invertebrates may be exposed to GM HO cotton, the altered fatty acid ratios in cottonseed and the introduced NPTII protein directly, through feeding on the plants and potentially, via the soil, when cotton tissues break down and are incorporated into the soil. Exposure may also occur indirectly, through eating other organisms that feed on the plants.

155. Relative exposure will be greatest for herbivorous species feeding on the cotton plants in particular those species that consume cottonseed. Sap feeders, such as aphids, will have minimal exposure to fatty acids or the introduced NPTII protein as the sap is primarily composed of sugars and mineral salts dissolved in water.

### **2.2.3 Microorganisms**

156. Microorganisms may be exposed to the GM cotton plants during growth or during decomposition of plant material, including the cottonseed. After harvest of lint and seed, the remaining cotton plant residues are typically tilled into the soil, so that soil microorganisms are likely to be exposed to the introduced NPTII protein as the residues are broken down. Exposure of organisms in soil to the introduced NPTII protein may also occur as a result of root exudations (Saxena et al. 1999; Stotzky 2000).

## **Section 2.3 Other sources of oleic acid and NPTII in the environment**

157. Oleic acid is an abundant component of all plant, fungal and animal cell membranes and fat storage structures. Oleic acid is also a component of some bacterial cell membranes.

158. The NPTII protein is widespread in the environment and in food chains, in naturally occurring kanamycin-resistant microorganisms found in soil and in mammalian digestive systems (Flavell et al. 1992a).

## **Section 2.4 Potential toxicity hazards for other organisms**

### **2.4.1 Livestock and wildlife, including mammals, birds and fish**

159. GM HO cotton has altered fatty acid ratios in the seed with elevated levels of oleic acid and reduced levels of palmitic and linoleic acids. Oleic acid is a common dietary component of all vertebrates and is not known to be toxic to any animal or bird. Although linoleic acid is an essential dietary component for mammals, it is still present in GM HO cottonseed (about 6% of total cottonseed fatty acids, compared with about 56% in non-GM cottonseed). Furthermore, cottonseed oil is unlikely to be the sole source of dietary linoleic fatty acid for any livestock or wildlife.

160. The NPTII protein is widespread in the environment and in food chains, in naturally occurring kanamycin-resistant microorganisms found in soil and in mammalian digestive systems (Flavell et al. 1992d). Acute oral toxicity studies in a range of mammalian species (mice, rats and rabbits) with NPTII protein has not demonstrated any adverse effects. Likewise, in trials with rats, quail or catfish fed cottonseed meal at 5 to 20 % of the diet, no significant differences in weight gain, feed conversion or gross necropsy were found for animals fed GM

cottonseed meal with NPTII protein compared to those fed non-GM cottonseed meal (Canadian Food Inspection Agency 1997).

161. Cottonseed or pollen is not expected to enter aquatic habitats in any significant quantity, limiting exposure of aquatic organisms. Irrigation practices (Good Management Practice of cotton industry) used by cotton growers in NSW retain irrigation water run-off, as well as the first 15 mm of storm water run-off, on farm to minimise the entrance of pesticide residues into natural waterways. The licence includes a requirement to separate cotton trials from natural waterways by at least 50 m.

162. GM HO cotton has not been tested in feeding trials and may therefore pose a low risk to wildlife. However, soybean meal from GM HO soybean was no more toxic than non-GM soybean in feeding trials of pigs and chickens (ANZFA 2001).

#### **2.4.2 Invertebrates**

163. Oleic acid is a common dietary component of seed eating insects. Oleic acid is a cell membrane component of all invertebrates. Elevated oleic acid in GM HO cottonseed is unlikely to be more toxic than non-GM cottonseed. However, it is not known how the altered fatty acid ratios may impact on the competitive ability of different species that consume cottonseed.

164. Pollinator species and various insects that feed on pollen will also have low exposure to the introduced NPTII protein, because of their expected much lower expression in pollen, relative to that in other plant tissues.

165. The direct effects of NPTII protein have not been tested on invertebrates. NPTII is a phosphorylating enzyme which does not possess any properties that distinguish it toxicologically from other phosphorylating enzymes present in microorganisms, plants and animals (FDA 1994). It is already widespread in the environment in bacteria. Thus the expression of this bacterial protein in plants is not likely to have any adverse toxic effects on invertebrates.

#### **2.4.3 Microorganisms**

166. Oleic acid is an abundant component of all plant and animal cell membranes and fat storage structures. Oleic acid is also a minor component of some bacterial cell membranes.

167. The NPTII protein is widespread in the environment, in kanamycin-resistant microorganisms naturally occurring in the soil, on plants and in animal guts. NPTII is a phosphorylating enzyme which does not possess any properties that distinguish it toxicologically from other phosphorylating enzymes present in microorganisms, plants and animals (FDA 1994). The function of this enzyme is the phosphorylation (inactivation) of the antibiotic neomycin (and the related kanamycin). In the environment, this enzyme is not likely to be active outside of living cells, as it requires specific chemical conditions for activity, including the availability of specific co-factors. Although antibiotic production by non-pathogenic bacteria has been implicated in suppression of some plant diseases (Brimecombe et al. 2001), no evidence for the involvement of neomycin or kanamycin has been found in a search of the scientific literature. Neither are these antibiotics used in agriculture for controlling soil borne disease. Thus the presence of NPTII in soil is not expected to impact on microbial populations or plant disease susceptibility. Furthermore, expression of NPTII in a variety of crop plants (for example, canola, corn, cotton, and tomato), over several years of agronomic performance testing and commercial cultivation, has not been linked to any increased

occurrence of disease. However, it is not known to what extent NPTII expression occurs amongst endophytic microorganisms. Therefore, expression of the NPTII protein in plants may impact on the diversity of endophytic bacteria and fungi.

### **Section 2.5 Other unintended effects**

168. A single plant gene, including genes inserted by genetic modification, can have an influence on multiple, sometimes unrelated, plant traits. This phenomenon is known as pleiotropy. Therefore it is necessary to evaluate genetically modified plants for unintended, pleiotropic effects of the inserted genes, such as changes in toxins or allergens.

169. Pleiotropic effects in GM HO cotton lines may arise from:

- disruption of gene function due to the site of insertion of the introduced genes into the cotton genome;
- secondary effects of altered oleic or linoleic acid content in GM HO cotton; and/or
- indirect effects on other parts of the same or different biochemical pathways.

170. Some of these unintended effects may alter traits that impact on the health and safety of other organisms. Data from plants grown under glasshouse conditions indicate that both HO lines, ?12-IR\*23 and ?12-IR\*124, have normal appearance and growth vigour, with no detectable differences compared to non-GM cotton (information supplied by the applicant). However, effects of the genetic modification on cottonseed composition, in particular, complete fatty acid profiles (including cyclopropenoids), protein content, and levels of gossypol, polyphenolic pigments, minerals and vitamins, have not been determined. A more comprehensive examination of plant characteristics is proposed as part of the field evaluation. (See Appendix 1, Section 8 for a more detailed consideration of pleiotropic effects.)

## **SECTION 3 CONCLUSIONS REGARDING TOXICITY TO OTHER ORGANISMS**

171. The proposed release is the first proof of concept trial of GM HO cotton to test its potential for producing cottonseed with high oleic acid when grown under Australian field conditions. GM HO cotton has not been tested in any animal feeding trials. There is limited data on expression and molecular characterisation of the introduced genes and information on altered plant properties is restricted to glasshouse trials. Nevertheless, it is considered that the risk of GM HO cotton being more toxic to other organisms than non-GM cotton is very low because:

- none of the GM cotton material from the release will be used for animal feed;
- expression of the introduced modified fatty acid desaturase results in altered fatty acid ratios in cottonseed but no novel fatty acids are expected to be produced;
- oleic acid is not known to be toxic to animals;
- oleic acid is widespread in the environment as a component of all plant and animal cells;
- the introduced NPTII protein is already widespread in the environment; and
- the NPTII protein is not known to be toxic to any organism.

172. The licence holder is required to report any adverse effects on human health and safety or the environment (for example, any indication of toxicity of the GM cotton for other organisms). Refer to Appendix 6 for the details of licence conditions.



## **APPENDIX 4 WEEDINESS**

173. 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 Appendix, risks posed by the proposed dealing to the environment are considered in relation to the potential for the GMO to become a significant weed.

174. There are numerous definitions of weeds including ‘a plant growing where it should not be’. Weeds are significant when they interfere with the intended use of the land they occupy, or adversely affect biodiversity. The impact of weeds on biodiversity may occur by direct competitive displacement or indirectly, by altering the local ecology or environment.

### **SECTION 1 NATURE OF THE POTENTIAL WEEDINESS HAZARD**

175. The possibility was considered that GM HO cotton might be harmful to the environment because of increased potential for weediness, either as a direct result of the genetic modification, or as a result of pleiotropic effects. Potentially, the GM HO cotton plants proposed for release could be at a selective advantage, which may enhance their weediness, if traits such as competitive ability, growth rate, seed production, seed dormancy, germination, persistence or potential for dispersal are affected due to the genetic modifications. Similarly, if resistance to pathogens or tolerance of herbivores is altered, the plants may have increased potential for weediness. If the GM HO cotton plants were to persist and spread in the environment as a weed, this could impact negatively on the environment, including loss of native biodiversity or adverse effects on agricultural systems.

### **SECTION 2 LIKELIHOOD OF THE WEEDINESS HAZARD OCCURRING;**

176. In assessing the likelihood of adverse impacts due to weediness of GM HO cotton, a number of factors were considered, including:

- the inherent weediness of non-GM cotton;
- the potential weediness of GM HO cotton; and
- the potential selective advantage conferred by the altered fatty acid trait or the introduced NPTII protein;
- any unintended effects that may influence the potential weediness of GM HO cotton.
- potential spread of GM HO cotton into the environment; and
- persistence of GM HO cotton at the site of release.

#### **Section 2.1 Inherent weediness of non-GM cotton**

177. Information on the weediness of non-GM cotton is included here to establish a baseline for comparison with GM HO cotton. Attributes of non-GM cotton associated with potential weediness are discussed in the document ‘The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia’ (OGTR 2002) that was produced in order to inform the risk assessment processes for licence applications involving GM cotton. This document can be accessed at [www.ogtr.gov.au](http://www.ogtr.gov.au). In summary, the document concludes that non-GM cotton is not a significant weed in Australia, because factors including soil moisture, nutrient limitation, frost, fire, herbivory, plant competition and roadside management practices limit the establishment and/or persistence of cotton seedlings.

178. One important element in the prediction of weediness is taxonomic relationship to recognized weeds, including its history of weediness in any part of the world (Bergelson et al. 1998; Panetta 1993; Pheloung 1995). Cotton has been grown for centuries throughout the world without any reports that it is a serious weed pest. Cotton is not considered to be a significant weed in Australia (Groves et al. 2000; Groves et al. 2002). Globally, there are about 50 species of *Gossypium* (Fryxell 1992; Craven et al. 1994) of which only one (*G. tomentosum*) is listed, in the USA, as a weed (Holm et al. 1997).

## **Section 2.2 Potential weediness of GM HO cotton**

179. Many of the characteristics associated with weediness are also important agronomic characteristics. Consequently these are assessed as part of the agronomic evaluations during the development of new cotton varieties, including GM varieties. Such characteristics have been thoroughly assessed in other field trials and commercial releases (refer to DIRs 005/2001, 006/2001, 009/2002 and 012/2002). However, there may be some possibility for, although considered low, pleiotropic effects of the genetic modification, which could potentially alter some aspect of the cotton biology that may affect weediness.

180. No unintended or secondary effects on agronomic characteristics, including no effects on fertility, have been observed in glasshouse trials of GM HO cotton (information supplied by the applicant). The applicant proposes to conduct continued evaluation of agronomic characteristics of GM HO cotton as part of the proposed field trial.

## **Section 2.3 Potential selective advantage conferred by the modified traits**

181. The potential for enhanced weediness of GM HO cotton compared to non-GM Coker 315 depends on the effects of the modified traits and whether these affect the spread and persistence of the GMO in the environment. The modified traits of GM HO cotton that may influence weediness include a change in fatty acid composition in cottonseed, antibiotic resistance and unintended pleiotropic effects.

### **2.3.1 Altered fatty acid composition in cottonseed**

182. In glasshouse trials GM HO cotton has altered fatty acid ratios in cottonseed with increased proportions of oleic acid. The changes appear to be restricted to developing cottonseed embryos, within the seed coat (information supplied by applicant). Altered fatty composition is most likely to affect germination properties, such as the optimal temperature for germination, proportion of seeds that are likely to germinate or the dormancy and persistence of HO cotton seeds.

183. If the proportion of GM HO cottonseeds that germinate increased, this may confer a selective advantage and enhance the GM cotton's potential weediness. However, the proportion of non-GM cotton seeds that germinate is naturally relatively high (>80%; see OGTR 2002) and any impact of the genetic modification, even if increasing that proportion, is likely to be minimal relative to non-GM cotton. Moreover, the germination of cotton seeds in the proposed release area of southern Australia is limited by physical variables such as temperature and soil moisture. The genetic modification to HO cotton is unlikely to affect its response to these germination cues.

184. If HO cottonseed were to show increased dormancy this may increase the potential for persistence of GM HO cotton progeny in the environment. However, the rate at which GM HO

cottonseeds germinate appears to be similar to that of non-GM cottonseed (information supplied by the applicant), thereby suggesting that the genetic modification has not affected seed dormancy. The applicant proposes to evaluate agronomic characteristics, which would include cottonseed and germination properties.

### **2.3.2 NPTII protein**

185. The NPTII protein could confer a selective advantage to GM cotton plants in the presence of a high concentration of neomycin or kanamycin. However, antibiotics are not applied to cotton crops and are not likely to be present in any environment where cotton grows. Thus the expression of NPTII is highly unlikely to confer any selective advantage on the GM HO cotton.

## **Section 2.4 Other unintended effects**

186. A single plant gene, including genes inserted by genetic modification, can have an influence on multiple, sometimes unrelated, plant traits. This phenomenon is known as pleiotropy. Therefore it is necessary to evaluate genetically modified plants for unintended, pleiotropic effects of the inserted genes, such as changes in agronomic characteristics related to weediness.

187. Pleiotropic effects in GM HO cotton lines may arise from:

- disruption of gene function due to the site of insertion of the introduced genes into the cotton genome;
- secondary effects of altered oleic or linoleic fatty acid content in GM HO cotton; and/or
- indirect effects on other parts of the same or different biochemical pathways.

188. Some of these unintended effects may alter traits that influence the potential for weediness. Data from plants grown under glasshouse conditions indicate that both HO lines, ?12-IR\*23 and ?12-IR\*124, have normal appearance and growth vigour, with no detectable differences compared to non-GM cotton (information supplied by the applicant). A more comprehensive examination of plant characteristics is proposed as part of the field evaluation. (See Appendix 1, section 8 for a more detailed consideration of pleiotropic effects.)

## **Section 2.5 Spread of GM HO cotton beyond the release site**

189. Natural dispersal of non-GM cottonseed in the environment is very limited because the lint-covered seed is too heavy to be dispersed by wind and is unattractive to potential animal vectors such as birds and grazing animals. While lint-covered seed could float for some distance along waterways, cotton farms generally offer very poor access to waterways beyond the on-farm irrigation systems. Thus there is only very limited potential for dispersal of cotton seeds without the assistance of human intervention. The genetic modification to HO cotton is highly unlikely to affect aspects of its seeds that may affect their natural dispersal.

190. After harvest, seed cotton may be dispersed beyond the limits of the farms where it is grown during transportation to ginning facilities. The escape of seed cotton during transportation presents an opportunity for the GM cotton to spread in the environment. Specific licence conditions have been imposed to require harvested material to be securely wrapped before transporting away from the release site so as to prevent seed cotton escaping into the environment.

191. The proposed dealing includes cultivation of GM HO cotton and harvesting all cottonseed for testing for the HO trait. Cottonseed produced in the proposed field trial will not be used for human food or stockfeed.

192. Cotton is primarily a self pollinating plant, with pollen that is unlikely to be dispersed by wind (OGTR 2002). However, insect vectors may transfer pollen to other cotton plants. Pollen dispersal studies have shown that outcrossing is localised around the pollen source and decreases significantly with distance (Umbeck et al. 1991; Llewellyn & Fitt 1996). The applicant proposes to limit outcrossing to non-GM cotton using pollen traps (information provided by the applicant). (Pollen flow is also discussed in Appendix 5, Section 1 in relation to gene transfer.)

193. Licence conditions have been imposed to limit dispersal of seed and pollen.

### **Section 2.6 Persistence of GM HO cotton at the release site**

194. Following harvest of the seed cotton from the release site, the remaining plant material will be slashed and incorporated into the soil. Some seed may fall to the ground during harvesting and may be incorporated into the soil. A soil seed bank represents an opportunity for the GMO to persist in the environment. Commercial cotton cultivars have little dormancy, meaning that seed will germinate with the arrival of favourable soil moisture and temperature conditions and, therefore, persistent soil seed banks are unlikely to be formed.

195. GM cotton volunteers are expected to germinate in the wet season following harvest. As the germination rate of GM HO cotton is comparable to that of non-GM cotton it appears that its seed dormancy has not been affected by the genetic modification (see Section 3.1 of this Appendix). Accordingly, GM HO cotton is not likely to form a soil seed bank, and no more likely to do so, than non-GM cotton.

196. Some GM HO cotton plants may persist in the environment as ‘volunteers’, either in the release area or, potentially, on roadsides along transportation routes. In southern Australia, however, volunteer cotton plants do not appear to form self-perpetuating populations and are not considered to be significant weeds because soil moisture, frosts and roadside vegetation management practices limit germination and the establishment of volunteer populations (OGTR 2002). The genetic modification to GM HO cotton is highly unlikely to alter its ability to respond to these environmental factors. Potential HO cotton volunteers are, therefore, unlikely to persist in the environment at higher frequencies than non-GM cotton volunteers.

197. Licence conditions have been imposed to require post harvest monitoring, and destruction of cotton volunteers to ensure that the GM cotton lines do not persist in the environment at the release site.

## **SECTION 3 CONCLUSIONS REGARDING WEEDINESS**

198. Cotton is not a significant weed in Australia and has low potential for dispersal by natural means. Major constraints on the weediness of non-GM cotton include soil moisture, nutrient availability, plant competition, herbivory, frost and fire.

199. The proposed release is the first proof of concept trial of GM HO cotton to test its potential for producing cottonseed with high oleic acid when grown under Australian field conditions. Information on altered plant properties is restricted to glasshouse trials.

Nevertheless, it is concluded that the risk of GM HO cotton becoming a significant weed is low because:

- The major constraints on weediness of non-GM cotton apply equally to GM HO cotton;
- the introduced genes in GM HO cotton are unlikely to affect these characteristics;
- the low potential for dispersal of cotton by natural means applies equally to GM HO cotton; and
- there is a low possibility for the genetic modification to have potential pleiotropic effects that could alter traits affecting weediness, therefore, the applicant proposes to collect data on agronomic characteristics associated with weediness during the field trial.

200. Furthermore, the release is of limited scale, restricted to a single growing season at one site of two hectares and control conditions have been imposed to ensure that the chance of spread or persistence of GM HO cotton is minimised.

201. However, further information on the impact of the genetic modifications on agronomic characteristics of GM cottons in Australian field conditions that may be indicative of potential weediness (eg. dormancy of seeds, competitive ability, susceptibility to natural enemies and spread within the environment) will be required before the Regulator could determine this conclusively. This information will be required before a larger scale or less stringently controlled release could be considered.

## APPENDIX 5 TRANSFER OF INTRODUCED GENES TO OTHER ORGANISMS

202. 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 risk management plan. This Appendix considers the likelihood that the introduced genes from GM HO cotton will be transferred to other organisms and the potential hazards that may be posed through such gene transfers.

203. The proposed field release is the first small scale, proof of concept trial of this GMO to test the potential for producing cottonseed with high oleic acid when growing GM cotton under Australian field conditions.

204. Gene transfer is the movement of genes between individuals. Within many species genes are routinely exchanged between individuals of successive generations through sexual reproduction. Hybrids can be produced between closely related species through sexual reproduction. For example, in plants cross-pollination of wheat and rye produces triticale, in animals fertilisation of a mare by a donkey produces a mule. Hybrid progeny may be fertile or sterile. Only fertile progeny have the possibility of passing on a new trait(s) and potential for introgression of the gene(s) involved into a population.

205. Without the application of gene technology, gene transfer is not commonly observed between distantly related species, except among bacteria and viruses. However gene transfer between sexually incompatible organisms can occur. Detailed examination of DNA sequence similarities reveals that ancestral plants have occasionally exchanged small DNA fragments with distantly related organisms. In general there seems to have been only very limited transfer of genes from plants to other types of organisms.

206. The likelihood of hazards arising from gene transfer is dependent on a number of factors that form a necessary chain, including:

- opportunity for gene transfer to occur such that the recipient organism is exposed to the genetic material in the form of pollen, plant cells or DNA; and
- occurrence of the genetic material being incorporated into the genome of the recipient organism at a site and in a configuration that allows the gene to be functional; and
- persistence of the transferred genetic material such that the newly modified organism is able to survive, reproduce and maintain the genetic modification; and
- significance of the transferred genetic material such that its presence and/or expression in the recipient organism will result in an adverse impact on human health and safety, or the environment.

207. A detailed assessment of the likelihood of gene transfer from GM cottons to other organisms is presented in the applications, DIR 005/2001, 006/2001, 008/2001, 009/2002, 012/2002, 015/2002, 016/2002, 017/2002, 021/2002 and 022/2002.

208. For ease of reference, the assessment of gene transfer to other organisms is presented in three sections:

- Section 1 details the nature and likelihood of genes introduced to HO cotton transferring to other plants, including other cotton crops;

- Section 2 details the nature and likelihood of genes introduced to GM HO cotton transferring to animals, including humans; and
- Section 3 details the nature and likelihood of genes introduced to GM HO cotton transferring to microorganisms, including bacteria and viruses.

209. Section 4 draws together the conclusions from these sections.

## **SECTION 1 GENE TRANSFER FROM GM HO COTTON TO OTHER PLANTS**

### **Section 1.1 Nature of the gene transfer hazard**

210. A number of factors influence the likelihood of gene flow occurring between sexually compatible plants. Pre-fertilisation considerations include physical proximity and pollen movement, synchrony of flowering, breeding system and floral characteristics and competitiveness of pollen. Post-fertilisation considerations include sexual compatibility, hybrid viability and fertility, viability and fertility of progeny through several generations of backcrossing and successful incorporation of the modified genes into the genome (introgression). For successful gene transfer to occur, all pre- and post-fertilisation requirements must be met. Failure to meet any one requirement will mean that gene transfer and incorporation of a gene or genes into the population after a hybridisation event cannot occur.

211. Transfer of the introduced genes or regulatory sequences to cultivated cotton plants are likely to present the same hazards and have the same potential impacts as the presence of the genes in the GM insecticidal cotton (see Appendices 2 - 4). However, the stability of the high oleic acid trait could vary depending on the effectiveness of modified  $\Delta 12$ -fatty acid desaturase expression to trigger gene silencing (see Appendix 1, Section 7) in different genetic backgrounds or in different crosses. One of the main aims in this pilot trial is to test the stability of the HO trait in two GM lines grown under field conditions.

212. Feral or naturalised cotton is the same species as cultivated cotton, however the morphological features in these populations suggest they are not derived from modern elite cultivars that have escaped from cultivation, but rather are derivatives of primitive cultigens introduced before 1900 (information provided by CSIRO). If gene transfer to feral cotton occurred, this may increase the likelihood that the genes would persist in the environment. The flow on impacts of such a transfer could depend on whether the introduced genes (modified *ghFAD2-1* and *nptII*) conferred any selective advantage to the feral cotton in its environment. (Refer to Appendix 4, Section 2 for discussion on this issue.)

213. In situations where gene transfer to other plants can occur, the hazards to the environment associated with any such transfers could be highly varied, broadly depending upon the resulting phenotype of the progeny, such as any alteration in survival or reproductive capacity.

### **Section 1.2 Potential hazards from the introduced genes**

#### **1.2.1 The modified *ghFAD2-1* (high oleic) gene**

214. The modified *ghFAD2-1* gene is expected to down regulate its own expression and any related genes, resulting in reduced levels of protein that convert oleic to linoleic acid. It is expected that the *FAD2-1* gene is closely similar in all cottons that belong to the genus *Gossypium*, which includes the cultivated species, *G. hirsutum* and *G. barbedense*. However, the modified *ghFAD2-1* gene will have more sequence differences compared to the *FAD2-1*

gene of plants from other genera. Consequently, gene silencing of the homologous *FAD2-1* gene in other plants may not be effective.

215. If gene silencing fails to be triggered, then expression of  $\omega$ -12-fatty acid desaturase in seed could be unimpaired or even increased compared to non-GM parent. The modified *ghFAD2-1* gene contains one complete copy of the gene that encodes a functional enzyme. Expression of  $\omega$ -12-fatty acid desaturase from both the introduced gene and the endogenous gene could increase the conversion of oleic to linoleic acid. Thus, lower proportions of oleic acid and higher proportions of linoleic acid could be produced in plants.

### **1.2.2 The *nptII* (antibiotic resistance) gene**

216. Plants expressing this gene could become resistant to the related antibiotics kanamycin and neomycin. This would only have an impact on plant survival if the antibiotic was used on the plants, or otherwise present in the environment of the plant, and were limiting its growth. Antibiotics are not generally applied to crops and would not limit their growth except at very high concentrations not found in the natural or agricultural environment. Expression of the *nptII* gene enabled selection of plant cells containing the genetic modification in the laboratory.

### **1.2.3 Promoters and other regulatory sequences**

217. If promoter and other regulatory sequences were to be transferred to other plants without the associated genes of the GM cottons, the expression of endogenous plant genes could be altered with unpredictable effects. The impact could be highly variable and would be dependent on any resulting phenotypic change induced.

218. The introduced nopaline synthase (*nos*) regulatory sequences are derived from a plant pathogen (*Agrobacterium tumefaciens*). However these sequences are not pathogenic in themselves nor do they cause any disease symptoms in GM plants.

219. All of the introduced regulatory sequences operate in the same manner as do endogenous plant regulatory elements. The transfer of endogenous regulatory elements to a new genetic context occurs naturally in all plant genomes and could also result in unpredictable effects. Thus the potential hazard from the introduced sequences is no different to that posed by sequence transfer from non-GM plants or sequence transfer occurring within the genome of a plant species.

## **Section 1.3 Likelihood of gene transfer from GM HO cotton to other plants**

220. The likelihood of gene transfer from cotton to other species has been thoroughly considered in the document “The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia”, available at [www.ogtr.gov.au](http://www.ogtr.gov.au), that was produced in order to inform the risk assessment processes for licence applications involving GM cotton, and assessed in other applications for the release of GM cottons into the Australian environment.

### **1.3.1 Gene transfer to cultivated cotton**

221. Cotton is primarily self pollinating, however in a cropping situation a low level of pollen transfer, by insect pollinators, to other nearby vegetation would be likely. For a detailed consideration of the likelihood of this occurring, including an overview of the pollination biology of cotton, see the document “The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia”.

222. *Gossypium barbedense* (pima cotton) is also used for commercial cotton production, but only to a very minor extent in Australia (Lake Tandou and Bourke, NSW). *G. hirsutum* and *G. barbedense* are closely related and hybridisation between the two species can occur, yielding fertile progeny. Hybrid progeny exhibit characteristics intermediate to the parents but typically with lower capacity to produce fruit. After several generations, progeny of the hybrids revert to the characteristics of one or other of the parents. *G. barbedense* and hybrids are not more weedy or difficult to control than is *G. hirsutum* (personal communication, Warwick Stiller & Greg Constable, CSIRO).

223. Pollination of volunteer cotton plants growing along the side of cultivated crops is likely to occur as readily as between crops.

### 1.3.2 Gene transfer to volunteer and naturalised cotton

224. Feral or naturalised cotton is the same species as cultivated cotton, however the morphological features in these populations suggest they are not derived from modern elite cultivars that have escaped from cultivation, but rather, are derivatives of primitive cultivars introduced before 1900 (information provided by CSIRO).

225. Naturalised cotton populations occur in northern Western Australia, Northern Territory, and Queensland, whereas the release site is in the cotton growing region of NSW. Therefore, gene transfer from non-GM cotton cultivated in NSW to naturalised cotton is thought to be unlikely because of the geographic distance.

226. Licence conditions have been imposed to limit cross-pollination to plants outside the release site (see Chapter 2 and Appendix 6 for details).

### 1.3.3 Gene transfer to native cottons

227. Australian flora contains 17 native *Gossypium* species. All of the Australian *Gossypium* species are diploids (C, G or K genomes), while the cultivated cottons are tetraploids (AD-genomes). The native species with highest potential for hybridising with *G. hirsutum* is *G. sturtianum*. Hybrids have been produced without application of plant hormones, when plants were planted in close proximity of each other. However these hybrids were sterile, effectively eliminating any potential for introgression of *G. hirsutum* genes into *G. sturtianum* populations. However, there is some potential for the hybrids to undergo gene doubling and restore fertility of hybrid offspring.

228. The centre of native *Gossypium* diversity in Australia is in northern Western Australia and the Northern Territory. Most of the Australian *Gossypium* species have limited distributions and occur at considerable geographic distance from cultivated cotton fields. Thus gene transfer from non-GM cotton cultivated in NSW to native cottons is prevented not only by genetic incompatibility but also by geographic constraints to cross-pollination (OGTR 2002).

### 1.3.4 Gene transfer to other plants

229. In contrast to sexually compatible plants, gene transfer between sexually unrelated plants is exceedingly rare. One exception is evidence for at least five exchanges of mitochondrial genes between distantly related plants, based on phylogenetic analysis of DNA sequences (Bergthorsson et al. 2003). The mechanism for the gene transfer is not known. At present there are no examples of plant nuclear or chloroplast gene transfers between sexually incompatible plants. The rapidly expanding sequence databases of plant genomes may reveal more examples

of gene transfer between plants. Nevertheless, sufficient sequence data already exists to indicate that any gene transfer between plants is likely to be detectable only on time scales of millions of years, requiring many thousands of generations before any one gene transfer event becomes fixed in a population.

230. The negligible rates of gene transfer between sexually incompatible plants probably reflect the limited opportunity for plant DNA uptake by other plants. Gene transfer between plants may even require intermediate vectors, such as bacteria, viruses, fungi, nematodes or insects. However, there may be opportunities for direct DNA exposure between plants through pollen spread, parasitism or mechanical damage.

## **SECTION 2 GENE TRANSFER FROM GM HO COTTON TO ANIMALS**

### **Section 2.1 Nature of the gene transfer hazard**

231. The potential hazards associated with the introduced genes in GM insecticidal and insecticidal/herbicide tolerant cottons transferring to animals, including humans, could be highly varied, broadly depending upon the phenotype of the recipient and any changes to the survival or reproductive capacity of it or its progeny.

### **Section 2.2 Potential hazards from the introduced genes**

#### **2.2.1 The modified *ghFAD2-1* (high oleic) gene**

232. If the modified *ghFAD2-1* gene was transferred to individual animal cells, the effect is uncertain. Animal cells also exhibit potential RNA based gene silencing, but they may respond differently to signals that are effective in plants. Therefore the modified *ghFAD2-1* gene may or may not be expressed. Nevertheless, the modified *ghFAD2-1* gene is unlikely to down regulate any endogenous *FAD* genes in animals because of insufficient sequence similarity between *FAD* genes and the lack of endogenous  $\Delta^{12}$ -fatty acid desaturase activity in animals.

233. The modified *ghFAD2-1* gene contains one complete copy of the gene that encodes a functional enzyme. If gene silencing fails to be triggered in animals, then expression of  $\Delta^{12}$ -fatty acid desaturase could occur and potentially convert oleic to linoleic acid in animal cells.

#### **2.2.2 The *nptII* (antibiotic resistance) gene**

234. Animals could express the antibiotic resistance gene, *nptII*, and gain the ability to degrade the antibiotics related to kanamycin and neomycin. If the transfer occurred to humans or other animals treated with these antibiotics, this may affect antibiotic treatment. However the gene product, the NPTII enzyme, would only be active within the transformed animal cells, where appropriate conditions and co-factors for activity exist, therefore interference with any antibiotic treatment is unlikely. Antibiotics do not control animals, so no selective advantage would result or lead to any significant effects, since this enzyme is naturally present in animals and their intestinal fauna.

#### **2.2.3 Promoters and other regulatory sequences**

235. If promoter and other regulatory sequences were to be transferred to animals without the associated genes of the GM cottons, the expression of endogenous genes could be altered with unpredictable effects. The impact could be highly variable and would be dependent on any resulting phenotypic change induced.

236. The introduced nopaline synthase (*nos*) regulatory sequences are derived from a plant pathogen (*Agrobacterium tumefaciens*). However these sequences are not pathogenic in themselves nor do they cause any disease symptoms in GM plants.

237. All of the introduced regulatory sequences operate in the same manner as do endogenous plant regulatory elements. The transfer of endogenous regulatory elements to a new genetic context could also result in unpredictable effects. Thus the likelihood of a hazard arising due to transfer of the introduced sequences is no different to that of sequence transfer from non-GM plants.

### **Section 2.3 Likelihood of gene transfer from GM HO cotton to animals**

238. In the case of multi-celled organisms such as humans or other animals (including nematodes), any rare uptake of plant DNA or RNA is likely to occur in non-reproductive (somatic) cells such as the lining of the gut when feeding on plant material, or at breaks in the skin. Potentially, this could result in a rare gene transfer to an individual cell (as yet unproven). However, even if such an event were shown to occur, the modified somatic cell with the introduced gene would not persist. Therefore, gene transfer from plants is probably limited primarily by the opportunity for plant DNA exposure to animal sex (germ line) cells and secondarily by the occurrence of stable integration into the genome.

239. At present the genome sequences of humans, and other animals and plants has not revealed any gene transfers between plants and animals. Conceivably, gene transfer could occur through a third agent, such as a bacterium. However, gene transfer from plants to bacteria and from bacteria to animals is exceedingly rare. For example, only about 40 bacterial genes have been transferred to the human lineage over a period of more than 100 million years (Salzberg et al. 2001).

240. No products from the GM cottons in the proposed field trials will be used for human food. Thus, the likelihood of gene transfer to humans is negligible. It is worth noting that cottonseed oil and linters are the only fraction of cotton plants used in human food. As these products are free of DNA, even if products of the GM cottons were approved by FSANZ for use in food, humans would not be exposed to GM cotton DNA via the digestive system, excluding the possibility of gene transfer to human cells in the gut.

241. GM cottonseed from the proposed field trials will not be fed to livestock. If GM cottonseed were fed to animals, the most significant route for entry of foreign DNA, as with humans, would be through food as it passes through the gastrointestinal tract. Thus, the likelihood of gene transfer to animals is negligible. The fate of DNA in the digestive tract of various animals has been studied and is discussed in the risk assessment for DIR 021/2002 and DIR 22/2002. These risk assessments concluded that the likelihood of transfer via food is extremely low, and not greater than the likelihood of transfer from other sources of the introduced genes in the environment.

## **SECTION 3 GENE TRANSFER FROM GM HO COTTON TO MICROORGANISMS**

### **Section 3.1 Nature of the gene transfer hazard**

242. In contrast to sexually incompatible plants and animals, certain microorganisms appear to have much greater opportunity for exposure to plant genetic material and well-documented mechanisms for efficient uptake and incorporation of foreign DNA into the recipient organism. For example, endophytic bacteria, fungi (eg mycorrhizal fungi), protists and plant viruses may

be routinely exposed to plant genetic material. Nevertheless, very few examples of plant gene transfer to microorganisms have been reported.

### **Section 3.2 Potential hazards from the introduced genes**

#### **3.2.1 The modified *ghFAD2-1* (high oleic) gene**

243. If the modified *ghFAD2-1* gene was transferred to individual microorganisms the effect is uncertain. The modified *ghFAD2-1* gene contains one complete copy of the gene that encodes a functional enzyme. Expression of  $\Delta^12$ -fatty acid desaturase could occur and potentially convert oleic to linoleic acid in bacteria, other microorganisms or the cells invaded by viruses. However, most bacterial cells have little oleic acid.

#### **3.2.2 The *nptII* (antibiotic resistance) gene**

244. Microorganisms with the *nptII* gene transferred from GM HO cotton could become resistant to the antibiotics (except for viruses, which are not susceptible to antibiotics). The consequences of this for human health and safety and the environment would depend on other characteristics of the microorganism (for example pathogenicity), the use and significance of the antibiotic(s) in clinical and/or veterinary practice and whether these antibiotics limit growth or survival of the microorganism in other circumstances.

245. Antibiotics may limit some microorganisms, either due to the use of antibiotic medicines or in some limited environmental situations where competing microorganisms produce antibiotics.

#### **3.2.3 Promoters and other regulatory elements**

246. If promoter and other regulatory sequences were to be transferred to microorganisms without the associated genes of the GM cottons, the expression of endogenous genes could be altered with unpredictable effects. The impact could be highly variable and would be dependent on any resulting phenotypic change induced.

247. The introduced nopaline synthase (*nos*) regulatory sequences are derived from a plant pathogen (*Agrobacterium tumefaciens*). However these sequences are not pathogenic in themselves nor do they cause any disease symptoms in GM plants.

248. All of the introduced regulatory sequences operate in the same manner as do endogenous plant regulatory elements. The transfer of endogenous regulatory elements to a new genetic context could also result in unpredictable effects. Thus the likelihood of a hazard arising due to transfer of the introduced sequences is no different to that of sequence transfer from non-GM plants.

### **Section 3.3 Other sources of the introduced genes in the environment**

249. Genes encoding  $\Delta^12$ -fatty acid desaturases are widespread in plants (including non-GM cotton), fungi and some bacteria. These genes could serve as a source for gene transfer as readily as the introduced modified *ghFAD2-1* gene.

250. The *nptII* gene was originally isolated from a mobile genetic element (transposon) found in the plasmids and chromosomes of common bacteria. Transposons are readily transferable between bacterial species in nature. The *nptII* gene is associated with transposon Tn5 and is observed in gram negative bacteria and *Pseudomonas sp.* While it is widely dispersed in the

environment, other genes that also confer resistance to neomycin and kanamycin are more common, and also readily transferable (Smalla et al. 1994; Belgian Biosafety Server 1999).

### **Section 3.4 Likelihood of gene transfer to microorganisms**

251. A detailed assessment of the likelihood of gene transfer from GM cottons to microorganisms is presented in the applications, DIR 005/2001, 006/2001, 008/2001, 009/2002, 012/2002, 015/2002, 016/2002, 017/2002, 021/2002 and 022/2002.

#### **3.4.1 Gene transfer to bacteria**

252. Bacteria reproduce by asexual means, producing clonal copies identical to the parent except for chance mutations. Nevertheless, more than 10% of some bacterial genomes have been acquired by non-clonal means. Gene transfer by non-clonal means appear to be a common feature in the evolutionary history of bacteria (Ochman et al. 2000; Kurland et al. 2003), although the actual amount is commonly overestimated (Daubin et al. 2003). Several mechanisms appear to be used by bacteria in lateral gene transfers, including the uptake and incorporation of naked DNA in the environment.

253. However, one salient feature of lateral gene transfers in bacteria is that almost all involve acquisitions from other bacteria. Very few gene acquisitions have been from other types of organisms. From gene sequence analyses of bacteria only very few plant genes have been transferred to bacteria and persisted over evolutionary time (Nielsen et al. 1998; Bertolla & Simonet, 1999). This includes genes of bacteria that possess intimate associations with plant material, such as plant bacterial pathogens, symbiotic bacteria involved in nitrogen fixation, and bacteria in the gut of animals that consume plants. In addition, bacteria residing on the plant surface can access nutrients leaking from the leaf or exuded from the root and they often aggregate in biofilms that can facilitate cell-to-cell contact and thereby possibly DNA transfer.

254. Some examples of gene transfer from plants to bacteria have been shown experimentally (Tepfer et al. 2003; De Vries et al. 2003). However, these examples are highly artificial and relied on special laboratory conditions. Using antibiotic selection to detect extremely rare events, *Acinobacter sp.* cells containing a defective copy of the neomycin resistance (*nptII*) gene (with 10 bp or 317 bp of DNA deleted) were observed to incorporate DNA from GM plants (sugarbeet, tomato, potato or oilseed rape) carrying the intact *nptII* gene, leading to restoration of neomycin resistance. Without the artificially introduced homology in the recipient strain, no uptake of DNA could be detected in *Acinobacter sp.* (Nielsen et al. 2000; De Vries et al. 2001a) or in *Pseudomonas stutzeri* (De Vries et al. 2001b).

255. Therefore, it would seem that bacteria have the opportunity and capacity for DNA acquisition from other organisms. However this is rarely observed in the case of gene uptake from plants, despite the common availability of plant DNA to bacteria in plants, in the guts of herbivores and in soil surrounding plants soil (Gebhard & Smalla 1999; Paget & Simonet 1994; Widmer et al. 1996; Widmer et al. 1997; Paget & Simonet 1997). Most likely this reflects strong negative selection pressures that constrain the bacterial lifestyle, such that other bacteria are more likely to be a source of advantageous genes.

#### **3.4.2 Gene transfer to viruses**

256. Plant viruses invade a plant cell and commonly interact with plant genetic material during their replication. Many hundreds of plant viral genomes have been characterised. Very rarely, plant gene sequences can be found in the viral genome (Mayo & Jolly, 1991; Dolja et al. 1994).

For example, a strain of one virus of potatoes has acquired a chloroplast sequence at the end of the viral genome (Mayo & Jolly, 1991).

257. In some cases it has been experimentally demonstrated that an introduced gene in GM plants can be transferred to plant viral genomes, both DNA and RNA (Greene & Allison, 1994; Wintermantel & Schoelz, 1996; Borja et al. 1999). The gene transfers were observed during viral infections under special circumstances that included:

- the introduced gene was of viral origin and function;
- the introduced gene had some sequences that are identical to the infecting virus; and
- the infecting virus was defective or at a selective disadvantage compared with other strains.

258. In addition, plant viruses commonly exchange genetic material between one another during replication, but less often between viruses with highly distinct gene sequences (Worobey & Holmes, 1999).

259. Taken together, these reports indicate that plant gene transfer to plant viral DNA and RNA genomes probably occurs during most infections at a very low, but measurable rate. However, selection may be a severe constraint on the survival, multiplication and competitiveness of any novel viruses that emerge. Therefore, gene transfer from plants to viruses may give rise to new viruses only if the plant gene is of viral origin.

260. Neither the modified  $\Delta 12$ -fatty acid desaturase and *nptII* genes, nor their regulatory elements, are of viral origin. The introduced genes are not expected to increase the susceptibility of GM HO cotton to virus infection or the rate of gene transfer from GM HO cotton to an infecting virus, compared with the negligible rates of gene transfer for non-GM cotton.

### **3.4.3 Gene transfer to other microorganisms**

261. The transfer of genes from plants to other microorganisms has been poorly studied. Uptake of DNA from the host plant by some fungi have been reported, including *Plasmidiophora 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). However, stable integration and inheritance of the plant DNA in the genome of these fungi has not been substantiated by experimental evidence (Nielsen 1998).

262. Gene transfer to other microorganisms, such as protists, has not been detected in the few genomes that have been characterised extensively.

## **SECTION 4 CONCLUSIONS REGARDING GENE TRANSFER TO OTHER ORGANISMS**

### **Section 4.1 Conclusions regarding gene transfer to other plants**

263. Cultivated cotton has limited outcrossing through insect pollinators. Therefore it is considered that some gene transfer from GM HO cotton to cultivated cotton (of both *G. hirsutum* and *G. barbedense*) is likely. However, the risks posed by any such gene transfers are low because:

- cotton is primarily self-pollinated;

- any introduced genes transferred to cultivated cotton would pose the same risks as the low risks posed by GM HO cotton; and
- gene transfer by pollen spread is very unlikely to extend beyond the proposed pollen trap.

264. Licence conditions have been imposed to limit gene transfer to cultivated cotton and a research requirement imposed to monitor the efficacy of gene transfer control measures (see Chapter 2, Appendix 6).

265. Although transfer of the introduced genes from GM cottons to naturalised cotton (both *G. hirsutum* and *G. barbedense*) may increase the likelihood that the genes could spread and/or persist in the environment, it is considered that the likelihood of a hazard arising through gene transfer to volunteer or naturalised cotton is low, because:

- cotton is primarily self-pollinated;
- any introduced genes transferred to cultivated cotton would pose the same risks as the low risks posed by GM HO cotton; and
- geographical isolation of the proposed trial site from populations of naturalised (feral) cotton.

266. The licence imposes conditions to limit gene transfer to naturalised cotton and a research requirement imposed to monitor the efficacy of gene transfer control measures (See Chapter 2, Appendix 6).

267. It is considered that the risk of gene transfer from GM cottons to native cotton species is negligible, because:

- infertility of hybrids between cultivated and native cottons due to sexual incompatibility; and
- geographical isolation of the proposed trial area from populations of native cottons.

268. It is considered that the risk of gene transfer from GM cottons to plants from other genera is negligible, because:

- inability to cross-pollinate and form viable hybrids due to sexual incompatibility.

#### **Section 4.2 Conclusions regarding gene transfer to animals, including humans**

269. It is considered that the risk of gene transfer from GM cottons to animals is negligible, because:

- none of the GM cotton material from the release will be used for human food or animal feed;
- FSANZ approval would be required before the GM materials could be used for human food;
- negligible opportunity for exposure of introduced genes to the germ-line of animals; and
- negligible occurrence of stable incorporation into the genome.

270. It should be noted that in the extremely unlikely event of such a transfer occurring, human health and safety and the environment are unlikely to be adversely effected.

### **Section 4.3 Conclusions regarding gene transfer to microorganisms**

271. It is considered that the risk of gene transfer from GM cottons to microorganisms is negligible, because:

- negligible persistence of rare transfer events due to strong negative selection pressures; and
- both of the introduced genes in GM HO cotton are already widespread in the environment.

## APPENDIX 6 LICENCE CONDITIONS

### **Note in relation to the Approval of Genetically Modified Foods for Human Consumption**

Food Standards Australia New Zealand (FSANZ, formerly the Australia New Zealand Food Authority), is responsible for human food safety assessment. Currently, CSIRO has not applied to FSANZ for evaluation of material from the GM cottons for use in human food. FSANZ approval would need to be obtained before any parts of the GM cottons such as oil and linters derived from GM cotton seed could be used as human food.

### **PART 1 GENERAL CONDITIONS**

#### **Duration of Licence**

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

#### **Holder of Licence**

2. The holder of this licence ('the licence holder') is CSIRO.

#### **Project Supervisor**

3. The licence holder must immediately notify the Regulator in writing if any of the contact details of the Project Supervisor change.

#### **No dealings with GMOs except as authorised by this licence**

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

#### **Permitted dealings**

5. The permitted dealings with the GMOs are to plant, grow and conduct experiments with the GMOs, and the possession, supply, use, transport and disposal of the GMOs for the purpose of any of the permitted dealings with the GMOs, or in the course of any of these dealings.

#### **Persons covered by this GMO licence**

6. The persons covered by this licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged to undertake any activity in connection with GMOs grown in the Location pursuant to this licence.

#### **Informing people of their obligations**

7. 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.
8. The licence holder must provide the Regulator, on the Regulator's written request, signed statements from persons covered by this licence that the licence holder has informed those people of the conditions of this licence that apply to them.

#### **Licence holder to notify of circumstances that might affect suitability**

9. 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**

10. The licence holder must inform the Regulator in writing 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 GMOs must allow auditing and monitoring of the dealing**

11. If a person is authorised by this licence to deal with GMOs 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**

12. The licence holder must, at all times, remain an accredited organisation in accordance with the Act and comply with its instrument of accreditation.

## **PART 2 INTERPRETATIONS AND DEFINITIONS**

This licence does not authorise dealings with GMOs that are otherwise prohibited as a result of the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

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.

In this licence:

**‘Act’** means the *Gene Technology Act 2000* (Cth) and equivalent provisions in corresponding State law.

**‘Clean’** (or **‘Cleaned’**), as the case requires, means:

- (a) in relation to a Location or other area, the Destruction of the GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants in that Location or area, to the reasonable satisfaction of the Regulator; or
- (b) in relation to Equipment, the removal and Destruction of the GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants from the Equipment, to the reasonable satisfaction of the Regulator.

**‘Cotton’** means plants of the species *Gossypium hirsutum* L.

**‘Destroy’**, (or **‘Destroyed’** or **‘Destruction’**) means, as the case requires, killed by one or more of the following methods:

- (a) stalk pulling; or
- (b) uprooting by ploughing; or
- (c) root cutting; or

- (d) burning; or
- (e) treatment with herbicide; or
- (f) hand weeding.

*Note: 'As the case requires' has the effect that, depending on the circumstances, one or more of these techniques may not be appropriate. For example, in the case of killing the remains of harvest of the GMOs, treatment of post harvest remains by herbicide would not be a sufficient mechanism.*

**'Equipment'** includes harvesters, seeders, storage equipment, transport equipment (eg bags, containers, trucks), clothing and tools.

**'GM'** means genetically modified.

**'GMOs'** means the genetically modified organism or organisms authorised for release by this licence.

**'Location'** means an area of land where the GMOs are planted and grown.

**'Material from Pollen Trap plants'** means seed, stubble, pollen or any GM material (including parts of a plant) that is derived from or produced by cotton from a Pollen Trap.

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

**'Natural Waterways'** means waterways other than irrigation channels, holding dams or storage ponds used to collect water runoff from irrigated areas.

**'OGTR'** means the Office of the Gene Technology Regulator.

**'Pollen Trap'** means an area of land, extending at least 20 metres in all directions from the outside edge of a Location.

**'Pollen Trap plant'** means Cotton from a Pollen Trap.

**'Regulator'** means the Gene Technology Regulator.

**'Volunteer plants'** means progeny of the GMOs or Pollen Trap plants, or regrowth of previous GM or non-GM cotton plants.

## **PART 3 SPECIFIC CONDITIONS**

### **Location and size of trial**

1. The permitted dealings with the GMOs may be undertaken during the Cotton growing season of 2003 – 2004 within the Shire of Narrabri, New South Wales.
2. The GMOs may only be grown at a single Location. That Location must not exceed 2 hectares.
3. At least 7 days prior to commencing to grow the GMOs at the Location, the Location's GPS coordinates and either a street address, or other directions to the Location, must be provided to the Regulator by notice in writing.
4. The licence holder must be able to access and control the Location to the extent necessary to comply with this licence, for the duration of the life of the licence.
5. Planting the GMOs at the Location must not commence after 31 May 2004.

### **Notification of planting of the GMOs**

6. The licence holder must provide notices in writing to the Regulator of the actual date or dates of commencement of planting of the GMOs at the Location (and Pollen Trap in respect of the Location) ('the actual planting date notice'). This notice must be provided within 7 days of commencement of planting of the GMOs at the Location.

### **Notification of commencement of flowering of the GMOs**

7. The licence holder must provide notices in writing to the Regulator in respect of each of the following:
  - (a) the short term forecasted date or dates of commencement of flowering of the GMOs at the Location (and Pollen Trap in respect of the Location) ('the short term forecast flowering date notice'). This notice must be provided at least 7 days, and not more than 20 days, prior to the forecasted date or dates of commencement of flowering set out in the notice; and
  - (b) the actual date or dates of commencement of flowering of the GMOs at the Location (and Pollen Trap in respect of the Location) ('the actual flowering date notice'). This notice must be provided within 7 days of commencement of flowering of the GMOs at the Location.

### **Notification of commencement of seed set of the GMOs**

8. The licence holder must provide notices in writing to the Regulator in respect of each of the following:
  - (a) the short term forecasted date or dates of commencement of seed set of the GMOs at the Location (and Pollen Trap in respect of the Location, if any) ('the short term forecast seed set date notice'). This notice must be provided at least 7 days, and not more than 20 days, prior to the forecasted date or dates of commencement of seed set, as set out in the notice;
  - (b) the actual date or dates of commencement of seed set of the GMOs at the Location (and Pollen Trap in respect of the Location, if any) ('the actual seed set date notice').

This notice must be provided within 7 days of commencement of seed set of the GMOs at the Location.

### **Notification of commencement of harvest of GMOs**

9. The licence holder must provide notices in writing to the Regulator in respect of each of the following:
  - (a) the short term forecasted date or dates of commencement of harvesting of the GMOs at the Location (and Pollen Trap in respect of the Location) ('the short term forecast harvest date notice'). This notice must be provided at least 7 days, and not more than 20 days, prior to the forecasted date or dates of commencement of harvesting set out in the notice; and
  - (b) the actual date or dates of commencement of harvesting of the GMOs at the Location (and Pollen Trap in respect of the Location) ('the actual harvest date notice'). This notice must be provided within 7 days of commencement of harvesting of the GMOs at the Location.

### **Pollen Traps**

10. A Pollen Trap must surround the Location.
11. The Pollen Trap must contain non-genetically modified Cotton that is grown in such a way as to reasonably promote a dense and vigorous growth and flowering of the non-genetically modified cotton at the same time as the GMOs.
12. The edge of the Pollen Trap that is farthest from the GMOs (the 'outer edge of the Pollen Trap') must not be within 50 metres of a Natural Waterway.
13. Pollen Trap plants must be handled and controlled as if they are the GMOs (ie subject to other applicable conditions elsewhere in this licence), and Material from Pollen Trap plants must be handled and controlled as if it is Material from the GMOs (ie subject to other applicable conditions elsewhere in this licence).
14. A Pollen Trap must be able to be accessed and controlled by the licence holder to an extent that is commensurate with the licence holder's rights to access and control the Location within it.

### **Research requirement**

15. The licence holder must, in consultation with the OGTR, conduct research on gene transfer from the GMOs to the Pollen Trap.
16. In accordance with any Guidelines issued by the Regulator in relation to annual reporting, the licence holder must provide the Regulator with a written report of the progress and results of the research. This report must accompany the annual report to be sent to the Regulator.

### **Harvest and post-harvest procedures**

17. If the GMOs or Pollen Trap plants are harvested, they must be harvested separately from any other Cotton.

18. If seed cotton harvested from the GMOs or from Pollen Trap plants is ginned, it must be ginned separately from any other Cotton.
19. The GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants, must not be used for stockfeed and must not enter the human food supply.
20. Following ginning, seed from the GMOs and Pollen Trap plants and must be:
  - (a) stored in a sealed container, within a locked facility that is signed so as to indicate that GM cotton seed is stored within the facility; or
  - (b) Destroyed by burning.
21. Any GM cotton seed obtained from ginning may only be transported to the extent necessary to store them, Destroy them by burning or take it to a facility certified by the Regulator to physical containment level PC2.
22. Cotton lint obtained from ginning of seed cotton harvested from the GMOs or Pollen Trap plants may be sold.

### **Cleaning – post harvest and generally**

23. Equipment, the Location or other area used pursuant to this licence in respect of GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants, must be Cleaned.
24. For the Location, either within 14 days of harvest of the GMOs or by 31 May 2004, whichever occurs first, the Location must be Cleaned.
25. If Equipment is Cleaned, the area in which the Equipment is Cleaned must also be Cleaned. (For the sake of clarity, it is not necessary for Equipment to be Cleaned only at a Location.)
26. Cleaning must occur immediately or as soon as practicable after the use and before it is used for any other purpose. (For example, if GM seed is ginned, the gin must be Cleaned immediately following its use and before any other Cotton is ginned.)
27. On the request of the Regulator, the Regulator must be provided with written documentation of the procedures in place to ensure continuing compliance with the Cleaning conditions in this licence.

### **Inspection**

28. Following Cleaning of the GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants at a Location or other area, the following places must be inspected for the existence of Volunteer plants:
  - (a) the Location;
  - (b) the Pollen Trap in respect of the Location;
  - (c) irrigation channels and drains through which water flows to and from the Location and the Pollen Trap; and

- (d) any areas used to Clean Equipment used in connection with the GMOs or to Destroy the GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants.
29. Inspection must be performed by a person who is able to recognise Volunteer plants.
30. All the places required to be inspected must be inspected at least once every 2 months for a period of at least 12 months that commences the last day of Cleaning of the Location.
31. The results of inspection activities must be recorded in a logbook. The logbook must be available on request for examination or photocopying by the OGTR. The findings of the inspections as recorded in the logbook must be included in the licence holder's annual report to the Regulator. The logbook must contain at least the following:
- (a) details of the areas inspected;
  - (b) details of the date of inspection;
  - (c) the names of the person or persons who undertook the monitoring and details of the experience, training or qualification that enabled them to recognise Volunteer plants;
  - (d) the number of Volunteer plants observed, if any;
  - (e) details of the development stages reached by the Volunteer plants, if any; and
  - (f) details of methods used to Destroy Volunteer plants, if any.
32. Any Volunteer plant identified must be Destroyed prior to the plant flowering.

#### **General conditions on use of Locations post-harvest**

33. If the GMOs are grown at the Location, no other Cotton plant of any kind may be grown at the Location or Pollen Trap in respect of the Location, after harvest of the GMOs or Pollen Trap plants until inspection obligations are completed.
34. If the GMOs are grown at the Location, no plants may be planted at the Location or Pollen Trap in respect of the Location until inspection obligations are completed unless:
- (a) the plants are grasses (grass pastures), cereals (cereal crops); or
  - (b) the plants are plants agreed to in writing by the Regulator; and
  - (c) the Regulator is satisfied that monitoring and Destruction of Volunteer plants prior to setting seed will not be adversely affected by the planting.

#### **Transportation of the GMOs, Material from GMOs, Pollen Trap plants and Material from Pollen Trap plants**

35. Subject to the conditions immediately below in respect of transportation, the GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants must be transported in accordance with the OGTR Guidelines for the Transport of GMOs (June 2001) issued by the Regulator.
36. Harvested GMOs, Pollen Trap plants, Material from the GMOs or Material from Pollen Trap plants may be transported to a ginning facility in a cotton module that is:
- (a) completely enclosed within 2 layers of tarpaulin ('double wrapped in tarpaulin'); or
  - (b) completely enclosed within a layer of tarpaulin inside a layer of shade cloth ('double wrapped in tarpaulin and shade cloth');

- (c) contained within an enclosed chain-bed truck specifically designed for the purpose of transporting cotton modules.

*(Explanatory note: double wrapping is intended to prevent dissemination of the enclosed material during transportation.)*

- 37. Cotton lint derived from GMOs and Pollen Trap plants from ginning is not subject to transportation conditions.
- 38. Every container used to transport the GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants must be labelled:
  - (a) to indicate that it contains genetically modified Cotton; and
  - (b) with telephone contact numbers for the licence holder and instructions to contact the licence holder in the event that the container is broken or misdirected.
- 39. The licence holder must have in place accounting procedures to verify whether the same quantity of GMOs, Material from the GMOs, Pollen Trap Plant or Material from Pollen Trap plants sent is delivered and must document routes, methods and procedures used for transportation of GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants.

### **Contingency Plans**

- 40. Within 30 days of the date of the commencement of this licence, a written Contingency Plan must be submitted to the Regulator detailing measures to be taken in the event of the unintended presence of the GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants, outside an area that must be inspected.
- 41. The Contingency Plan must include details of procedures to:
  - (a) ensure the Regulator is notified immediately if the licence holder becomes aware of the event;
  - (b) destroy any of the GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants; and
  - (c) inspect and Destroy any Volunteer plants that may exist as a result of the event.
- 42. The Contingency Plan must be implemented in the event that the unintended presence of the GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants is discovered outside an area that must be inspected.

### **Compliance Management Plan**

- 43. Prior to growing the GMOs, a written Compliance Management Plan must be provided to the Regulator. The Compliance Management Plan must describe in detail how the licence holder intends to ensure compliance with these conditions and document that compliance.

### **Reporting**

- 44. The licence holder must provide the Regulator with a written report within 90 days of the anniversary of this licence, in accordance with any Guidelines issued by the Regulator in relation to annual reporting. This report must include information on any adverse impacts

on human health and safety or the environment, caused as a result of the GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants.

**Testing methodology**

45. The licence holder must provide a written instrument to the Regulator describing an experimental method that is capable of reliably detecting the presence of the GMOs and the presence of the genetic modifications described in this licence in a recipient organism. The instrument must be provided within 12 months of the issuing of this licence.

**Use of GMOs, Material from the GMOs, Pollen Trap plants and Material from Pollen Trap plants**

46. The licence holder must ensure that the GMOs, Material from the GMOs, Pollen Trap plants or Material from Pollen Trap plants are not consumed by humans or used in manufacture of animal feed or therapeutics.

## **APPENDIX 7 LEGISLATIVE REQUIREMENTS FOR ASSESSING DEALINGS INVOLVING INTENTIONAL RELEASES**

### **SECTION 1 THE REGULATION OF GENE TECHNOLOGY IN AUSTRALIA**

272. 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).

273. The Act establishes a statutory officer, the Gene Technology Regulator (the Regulator), to administer the legislation and make decisions under the legislation.

274. The Regulator is supported by the Office of the Gene Technology Regulator (OGTR), an Australian Government regulatory agency located within the Health and Ageing portfolio.

275. 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).

276. 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.

277. Detailed information about the national regulatory system and the gene technology legislation is also available from the OGTR website ([www.ogtr.gov.au](http://www.ogtr.gov.au)).

### **SECTION 2 THE LICENCE APPLICATION**

278. 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.

279. 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.

280. 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.

281. 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**

282. 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 Australian Government agencies (Regulation 9 of the *Gene Technology Regulations 2001* refers);
- the Environment Minister; and
- relevant local council(s) where the release is proposed.

283. 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.

284. 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**

285. 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.

286. 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.

287. 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.

288. 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

289. 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.

290. 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.

291. 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.

292. 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

293. 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.

294. The Regulator must also have regard to any policy guidelines issued by the Ministerial Council that relate to risks to human health and safety and the environment, or the management of such risks. At this time no policy guidelines have been issued.

295. The Regulator also must not issue a licence if this would be inconsistent with a policy principle issued by the Ministerial Council. 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 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.

296. 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.

297. The Regulator carefully considers all of this information which is supplied in a declaration signed by licence applicants.

298. 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.

299. If a licence is issued, the Regulator may impose licence conditions (Section 62 of the Act). For example, conditions may be imposed to:

- limit the scope of the dealings;
- require documentation and record-keeping;
- require a level of containment;
- specify waste disposal methods;
- manage risks posed to the health and safety of people, or to the environment;
- require data collection, including studies to be conducted;
- limit the geographic area in which the dealings may occur;
- require contingency planning in respect of unintended effects of the dealings; and
- limit the dissemination or persistence of the GMO or its genetic material in the environment.

300. 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.

301. 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 unintended problems.

## APPENDIX 8 SUMMARY OF PUBLIC SUBMISSIONS ON THE RISK ASSESSMENT AND RISK MANAGEMENT PLAN

**Submission from:** A: agricultural organisation; I: individual.

**Issues raised/consideration:** D: insufficient data/evidence; FC: food chain; FSANZ: food safety and labelling; H: human health and safety; L: labeling; MA: markets; OSA: outside scope of the assessment; RA: risk assessment; RM: risk management; SEG: segregation.

Sub. No:	Type	Summary of issues raised	Issue	Consideration of issue
1	A	Demand for clean and natural produce is reflected in the rapid growth of the global organic market, currently valued at \$40 billion.	FC, MA	FSANZ, OSA
		Uptake of GMOs and their use in the food chain must be accountable, with appropriate labelling of all GMO products and accurate identification to ensure product integrity and consumer confidence.	FC, L	FSANZ, OSA
		Cottonseed meal comprises up to 5% of pig's diet in Australia, and could safely be raised to 20% during feed shortage. Thus, the introduction of GM cotton into the food chain holds strong implications for the pork industry.	FC, MA	FSANZ, OSA
		There are risks through partial or complete rejection of animals fed GM products. Australian pork exporters are now required to provide written assurance to Japanese customers that the diets of their pigs are GMO free.	FC, MA	FSANZ, OSA
		Introduction of GM cotton is likely to increase the amount of cotton seed meal for use in pig diets provided any changes result in cheaper prices. This will offer improvements in ingredient supply and could enhance competitiveness in markets where GMOs are not an issue. However in our major Asian markets ... unlikely to provide a competitive advantage.	FC, MA	FSANZ, OSA
		Imperative that tracking, tracing an identity preservation issues are addressed to avoid contamination and ensure that the introduction of GMOs would not impede our export expansion internationally nor negatively impact on our domestic markets.	FC, L, MA, SEG	FSANZ, OSA
		While it is beyond the scope of the current RARMP, further research needs to be conducted aimed at the prospects of improving and expanding on-farm food safety systems and to develop effective tracking and tracing and identity preservation systems. If the benefits, if any, are to be realised, ... produce must be traced from its point of origin through to its final destination ... through to the end of the production process.	D, FC, L, SEG, MA	FSANZ, OSA
		Need for the cotton growers and policy makers to consider identifying appropriate risk management, tracking and tracing, communication strategies and appropriate labelling, for not just the cotton fibre end product but also for the GM inputs to other industries.	FC, L, SEG, MA	FSANZ, OSA
2	I	Management conditions to limit the spread are a vague idea ... there is no limiting the spread, future life, or mutation of GMOs. Low risk to public health and safety, or the environment is unacceptable to the majority of Australians.	RA, RM	App 2-6

## APPENDIX 9 REFERENCES

- ANZFA (2000). Draft risk analysis report, application A355: Food produced from glyphosate-tolerant cotton line 1445. Australia New Zealand Food Authority, Canberra, Australia.
- ANZFA (2001). Food derived from glyphosate-tolerant cotton line 1445 - A safety assessment. Australia New Zealand Food Authority, Canberra, Australia.
- ANZFA (2002). Draft assessment report (Full assessment - S.15) Application A436: Oil and linters derived from insect-protected cotton containing event 15985. Report No. Full assessment - S.15 Application A436, Australia New Zealand Food Authority, Canberra, Australia.
- Astwood, J.D., Leach, J.N., Fuchs, R.L. (1996). Stability of food allergens to digestion *in vitro*. *Nature Biotechnology* **14**: 1269-1273.
- Beck, E., Ludwig, G., Auerswald, E.A., Reiss, B., Schaller, H. (1982). Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn5. *Gene* **19**: 327-336.
- Belgian Biosafety Server (1999). Types of antibiotics and related resistance genes. (<http://www.antibioresistance.be/ARmenu.html>).
- Berberich, S.A., Leimgruber, R.M., and Regan, G.J. (1993). Preparation and verification of dose for a mouse acute oral toxicity study with neomycin phosphotransferase II protein (NPTII), study ML-91-409. Report No. MSL 13277, Monsanto Company.
- Bergelson, J., Purrington, C.B., Wichmann, G. (1998). Promiscuity in transgenic plants. *Nature* **395**: 25.
- Bevan, M. (1984). Binary *Agrobacterium* vectors for plant transformation. *Nucleic Acids Research* **12**: 8711-8721.
- Brimecombe, M.J., De Leij, F.A., Lynch, J.M.(2001). The effect of root exudates on rhizosphere microbial populations. In R Pinton, Z Varanini, P Nannipieri, eds "The rhizosphere: biochemistry and organic substances at the soil-plant interface". Marcel Dekker, Inc., New York, USA, pp 95-140.
- Bryngelsson, T., Gustafson, M., Green, B., Lind, C. (1988). Uptake of host DNA by the parasitic fungus *Plasmodiophora brassicae*. *Physiological and Molecular Plant Pathology* **33**: 163-171.
- Buhariwalla, H., Mithen, R. (1995). Cloning of a *Brassica* repetitive DNA element from resting spores of *Plasmodiophora brassicae*. *Physiological and Molecular Plant Pathology* **47**: 95-101.
- Canadian Food Inspection Agency (1997). Decision Document 97-21: Detemination of the safety of cotton lines with Roundup Ready genes (*Gossypium hirsutum* L.). Report No. 97-21 , Plant health and Produciton Division, Plant Biotechnology Office,
- Craven, L. A., Stewart, J. M., Brown, A. H. D., and Grace, J. P. (1994). Challenging the future; the Australian wild species of *Gossypium*. pp. 278-281.

De Vries, J., Meier, P., Wackernagel, W. (2001b). The natural transformation of the soil bacteria *Pseudomonas stutzeri* and *Acinetobacter* sp. by transgenic plant DNA strictly depends on homologous sequences in the recipient cells. *FEMS Microbiology Letters* **195**: 211-215.

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EPA (1994). Neomycin phosphotransferase II; tolerance exemption. *Federal Register* **59**: 49351-49353.

FDA (1994). Secondary food additives permitted in food for human consumption; food additives permitted in feed and drinking water of animals; aminoglycoside 3'-phosphotransferase II; final rule. Report No. 59, United States Food and Drug Administration, Washington, USA.

FDA (1998). Guidance for Industry: Use of antibiotic resistance marker genes in transgenic plants. U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Premarket Approval, <http://vm.cfsan.fda.gov/~dms/opa-armg.html>.

Felsot, A.S. (2000). Insecticidal genes. Part 2: Human health hoopla. *Agrichemical and environmental news* **168**: 1-7.

Flavell, R.B., Dart, E., Fuchs, R.L., Fraley, R.T. (1992d). Selectable marker genes: safe for plants? *Bio/Technology* **10**: 141-144.

Flavell, R.B., Dart, E., Fuchs, R.L., Fraley, R.T. (1992a). Selectable marker genes: safe for plants? *Bio/Technology* **10**: 141-144.

Flavell, R.B., Dart, E., Fuchs, R.L., Fraley, R.T. (1992b). Selectable marker genes: safe for plants? *Bio/Technology* **10**: 141-144.

Flavell, R.B., Dart, E., Fuchs, R.L., Fraley, R.T. (1992c). Selectable marker genes: safe for plants? *Bio/Technology* **10**: 141-144.

Fryxell, P.A. (1992). A revised taxonomic interpretation of *Gossypium* L. (Malvaceae). *Rheedea* **2**: 108-165.

Fuchs, R.L., Astwood, J.D. (1996). Allergenicity assessment of foods derived from genetically modified plants. *Food Technology* **50**: 83-88.

Fuchs, R.L., Ream, J.E., Hammond, B.G., Naylor, M.W., Leimgruber, R.M., Berberich, S.A. (1993b). Safety assessment of the neomycin phosphotransferase II (NPTII) protein. *Biotechnology (NY)* **11**: 1543-1547.

Fuchs, R.L., Ream, J.E., Hammond, B.G., Naylor, M.W., Leimgruber, R.M., Berberich, S.A. (1993a). Safety assessment of the neomycin phosphotransferase II (NPTII) protein. *Biotechnology (NY)* **11**: 1543-1547.

Gebhard, F., Smalla, K. (1999). Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer abstract no. 3). *Fems Microbiology Ecology* **28**: 261-272

- Groves, R.H., Hosking, J.R., Batianoff, D.A., Cooke, D.A., Cowie, I.D., Keighery, B.J., Rozefelds, A.C., and Walsh, N.G. (2000). The naturalised non-native flora of Australia: its categorisation and threat to native plant biodiversity. Unpublished report to Environment Australia by the CRC for Weed Management Systems.
- Groves, R.H., Hosking, J.R., Cooke, D.A., Johnson, R.W., Lepschi, B.J., Mitchell, A.A., Moerkerk, M., Randall, R.P., Rozefelds, A.C., and Waterhouse, B.M. (2002). The naturalised non-native flora of Australia: its categorisation and threat to agricultural ecosystems. Unpublished report to Agriculture, Fisheries and Forestry Australia. CRC for Weed Management Systems,
- Hoffman, T., Golz, C., Schieder, O. (1994). Foreign DNA sequences are received by a wild-type strain of *Aspergillus niger* after co-culture with transgenic higher plants. *Current Genetics* **27**: 70-76.
- Holm, L., Doll, J., Holm, E., Pancho, J., Herberger, J. (1997). World weeds. Natural histories and distribution. John Wiley and Sons, Inc, USA.
- Kimber, I., Kerkvliet, N.L., Taylor, S.L., Astwood, J.D., Sarlo, K., Dearman, R.J. (1999). Toxicology of protein allergenicity: prediction and characterization. *Toxicological Sciences* **48**: 157-162.
- Klee, H.J., Rogers, S.G. (1989). Plant gene vectors and genetic transformation: plant transformation systems based on the use of *Agrobacterium tumefaciens*. *Cell Culture and Somatic Cell Genetics of Plants* **6**: 1-23.
- Leffler, H.R., Tubertini, B.S. (1976). Development of cotton fruit: accumulation and distribution of mineral nutrients. *Agronomy Journal* **68**: 858-861.
- Levy, S.B., Marshall, B., Schluederberg, S., Rowse, D., Davis, J. (1998). High frequency of antimicrobial resistance in human fecal flora. *Antimicrobial Agents and Chemotherapy* **32**: 1801-1806.
- Llewellyn, D., Fitt, G. (1996). Pollen dispersal from two field trials of transgenic cotton in the Namoi valley, Australia. *Molecular Breeding* **2**: 157-166.
- Metcalf, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L., Fuchs, R.L. (1996). Assessment of the allergenic potential of foods derived from genetically engineered crop plants. *Critical Reviews in Food Science and Nutrition* **36(S)**: S165-S186.
- Nielsen, K.M. (1998). Barriers to horizontal gene transfer by natural transformation in soil bacteria. *APMIS* **106**: 77-84.
- Nielsen, K.M., van Elsas, J.D., Smalla, K. (2000). Transformation of *Acinetobacter* sp strain BD413(pFG4 Delta nptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. *Applied and Environmental Microbiology* **66**: 1237-1242.
- OGTR (2002). The Biology and Ecology of Cotton (*Gossypium hirsutum*) in Australia.
- Paget, E., Simonet, P. (1994). On the track of natural transformation in soil. *Fems Microbiology Ecology* **15**: 109-118.

- Paget, E., Simonet, P. (1997). Development of engineered genomic DNA to monitor the natural transformation of *Pseudomonas stutzeri* in soil-like microcosms. *Canadian Journal of Microbiology* **43**: 78-84.
- Panetta, F.D. (1993). A system of assessing proposed plant introductions for weed potential. *Plant Protection Quarterly* **8**: 10-14.
- Pheloung, P.C. (1995). Determining the weed potential of new plant introductions in Australia. Department of Agriculture, Perth, Australia.
- Saxena, D., Flores, S., Stotzky, G. (1999). Insecticidal toxin in root exudates from *Bt* corn. *Nature* **402**: 480.
- Sims, S.R., Berberich, S.A., Nida, D.L., Segalini, L.L., Leach, J.N., Ebert, C.C., Fuchs, R.L. (1996). Analysis of expressed proteins in fibre fractions from insect-protected and glyphosate-tolerant cotton varieties. *Crop Science* **36**: 1212-1216.
- Smalla, K., Gebhard, F., van Elsas, J. D., Matzk, A., and Schiemann, J. (1994). Bacterial communities influenced by transgenic plants. Jones, D. D. eds, Division of Agriculture and Natural Resources, University of California, Oakland, USA. pp. 157-167.
- Stotzky, G. (2000). Release, persistence, and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis*.
- Taylor, S.L., Lehrer, S.B. (1996). Principles and characteristics of food allergens. *Critical Reviews in Food Science and Nutrition* **36**: S91-S118.
- Umbeck, P.F., Barton, K.A., Nordheim, E.V., McCarty, J.C., Parrot, W.L., Jenkins, J.N. (1991). Degree of pollen dispersal by insects from a field test of genetically engineered cotton. *Journal of Economic Entomology* **84**: 1943-1950.
- Widmer, F., Seidler, R.J., Donegan, K.K., Reed, G.L. (1997). Quantification of transgenic plant marker gene persistence in the field. *Molecular Ecology* **6**: 1-7.
- Widmer, F., Seidler, R.J., Watrud, L.S. (1996). Sensitive detection of transgenic marker gene persistence in soil microcosms. *Molecular Ecology* **5**: 603-613.