Australian Government
Department of Health and Ageing
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for **DIR 095**

Limited and controlled release of sugarcane genetically modified for altered plant growth, enhanced drought tolerance, enhanced nitrogen use efficiency, altered sucrose accumulation, and improved cellulosic ethanol production from sugarcane biomass

Applicant: BSES Limited

July 2009

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Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of licence application (DIR 095) from BSES Limited (BSES). The licence authorises dealings involving the limited and controlled release of up to 12,500 lines[1] of genetically modified (GM) sugarcane into the environment.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether to issue a licence to deal with a genetically modified organism (GMO). The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with the *Risk Analysis Framework* and finalised following consultation with a wide range of experts, agencies and authorities and the public[2].

The application

BSES applied for a licence for dealings involving the intentional release of up to 12,500 lines of GM sugarcane on a limited scale and under controlled conditions. The sugarcane lines will be genetically modified to alter plant growth, enhance drought tolerance, enhance nitrogen use efficiency, alter sucrose accumulation or improve cellulosic ethanol production from sugarcane biomass. The trial will place at six BSES stations in the Queensland local government areas of Moreton Bay, Bundaberg, Mackay, Burdekin and Cairns, on a maximum total area of 21 ha, between August 2009 and August 2015.

The GM sugarcane lines will contain one or more genes or gene fragments from 22 genes derived from a range of plant and bacterial species. Some of the GM sugarcane lines will be modified to express proteins encoded by the introduced genes and some will contain genes or parts of genes designed to suppress the function of endogenous sugarcane genes. In addition, each GM sugarcane line will contain one or two genes encoding antibiotic resistance selectable marker genes used during their initial development in the laboratory.

The purpose of the trial is to evaluate agronomic properties of the GM sugarcane lines grown under field conditions. Promising lines will be selected for crossing under controlled conditions to other GM sugarcane lines or non-GM sugarcane cultivars for possible future commercial development (subject to additional approvals). The GM sugarcane will not be used for human food or animal feed.

BSES proposed a number of controls to restrict the dissemination and persistence of the GM sugarcane lines and their introduced genetic materials in the environment that were considered during the evaluation of the application.

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[1] The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

[2] More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030 or at <http://www.ogtr.gov.au/>), and in the Regulator's *Risk Analysis Framework* (OGTR 2007) at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

Confidential Commercial Information

Some details, including the identities of several genes and regulatory sequences, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

Risk assessment

The risk assessment took into account information in the application (including proposed containment measures), relevant previous approvals, current scientific knowledge and advice relating to risks to human health and safety and the environment provided in submissions received during consultation on the RARMP. No new risks to people or the environment were identified from the advice received on the consultation RARMP.

A **hazard** identification process was used in the first instance to determine potential pathways that might lead to harm to people or the environment as a result of gene technology.

Nine events were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

A **risk** is only identified when a hazard is considered to have some chance of causing harm. Events that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

The characterisation of the nine events in relation to both the magnitude and probability of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment.

Any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM sugarcane lines into the environment are considered to be **negligible**. Hence, the Regulator considers that the dealings involved in this limited and controlled release **do not pose a significant risk** to either people or the environment.

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment. As none of the nine events characterised in the risk assessment are considered to give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is considered to be **negligible**.

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, conditions are imposed to restrict the dissemination and persistence of the GMOs and their genetic material in the environment and to limit the release to the size and locations requested by the applicant as these were important considerations in establishing the context for assessing the risks. The context for assessing the risks may change substantially over the 15 year period proposed by the applicant, potentially impacting upon the conclusions of the risk assessment. Therefore, the imposed licence conditions limit the duration of the release to six years.

The licence conditions require BSES to **limit** the release to a total area of 21 ha at six BSES stations between August 2009 and August 2015. The **control** measures include containment

provisions at the trial site, preventing the use of GM plant materials in human food or animal feed; destroying GM plant materials not required for further studies; transporting GM plant materials in accordance with the Regulator's transportation guidelines; and conducting post-harvest monitoring at the trial site to ensure all GMOs are destroyed.

Conclusions of the RARMP

The risk assessment concluded that this limited and controlled release of up to 12,500 lines of GM sugarcane on a maximum total area of 21 ha over 15 years in the Queensland local government areas of Moreton Bay, Bundaberg, Mackay, Burdekin and Cairns poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to restrict the release to the size and locations requested by the applicant as these were important considerations in establishing the context for assessing the risks. The context for assessing the risks may change substantially over the 15 year period proposed by the applicant, potentially impacting upon the conclusions of the risk assessment. Therefore, the imposed licence conditions limit the release to six years, rather than the 15 years proposed by the applicant.

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Abbreviations

the Act	*Gene Technology Act 2000*
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
bla	β-lactamase
BSES	BSES Limited, formerly the Bureau of Sugar Experiment Stations
CCI	Confidential Commercial Information as declared under section 185 of the *Gene Technology Act 2000*
CCS	commercial cane sugar
DIR	Dealings Involving intentional Release
DNA	Deoxyribonucleic acid
DRE	Drought responsive element
EST	Expressed sequence tag
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
GM	Genetically Modified
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
ha	Hectare
HGT	Horizontal gene transfer
HvGA20ox	*Hordeum vulgare* gibberellin 20-oxidase
LGA	Local government area
m	metre
mm	millimetre
mRNA	Messenger Ribonucleic Acid
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
Nos	Nopaline synthase
nptII	Neomycin phosphotransferase II
Ocs	Octopine synthase
OGTR	Office of the Gene Technology Regulator
OsDREB1A	*Oryza sativa* Drought-responsive element-binding protein1A
OsTB1	*Oryza sativa* Teosinte branched1
PC2	Physical containment level 2
PcGA2ox-1	*Phaseolus coccineus* gibberellin 2-oxidase-1
PCR	Polymerase Chain Reaction
PR	Promoter
RARMP	Risk Assessment and Risk Management Plan
RbcS	Rubisco small subunit
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
RNAi	RNA interference
Rubisco	ribulose-1’5’-bisphosphate carboxylase/oxygenase
SA	Sugar accumulation gene
ShTB1	*Saccharum* hybrid Teosinte branched1
T-DNA	Transfer DNA
TGA	Therapeutic Goods Administration

Tml	Tumour morphology large
Ubi1	Ubiquitin1
uidA	β-glucuronidase
US EPA	United States Environmental Protection Agency
VPE	Vacuole processing enzyme
WUE	Water use efficiency gene
ZmDof1	*Zea mays* DNA-binding with one finger 1

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Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of application (DIR 095) from BSES Limited (BSES). The licence authorises dealings involving the limited and controlled release of up to 12,500 lines[3] of genetically modified (GM) sugarcane into the environment.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Regulator before making a decision whether to issue a licence to deal with a genetically modified organism (GMO). The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with the *Risk Analysis Framework* and finalised following consultation with a wide range of experts, agencies and authorities and the public[4].

The application

BSES has applied for a licence for dealings involving the intentional release of up to 12,500 lines of GM sugarcane on a limited scale and under controlled conditions. The GM sugarcane lines will be genetically modified to alter plant growth, enhance drought tolerance, enhance nitrogen use efficiency, alter sucrose accumulation or improve cellulosic ethanol production from sugarcane biomass. The trial will take place at six BSES stations in the Queensland local government areas of Moreton Bay, Bundaberg, Mackay, Burdekin and Cairns, on a maximum total area of 21 ha. BSES applied to conduct the trial between June 2009 and June 2024.

The GM sugarcane lines will contain one or more genes or gene fragments from 22 genes derived from a range of plant and bacterial species. Some of the GM sugarcane lines will be modified to express proteins encoded by the introduced genes. Others will contain genes or parts of genes designed to suppress the function of endogenous sugarcane genes, through a mechanism known as gene silencing or RNA interference (RNAi). In addition, each GM sugarcane line will contain one or two genes encoding antibiotic resistance selectable marker genes used during their initial development in the laboratory.

The applicant aims to modify plant growth by expression of gibberellin biosynthetic enzymes from runner bean and barley to modify plant height, and by expression or silencing of a transcription factor from rice or sugarcane which controls tillering. Enhanced drought tolerance is expected as a result of the expression of genes from a common plant and a common bacterium involved in plant hormone biosynthesis, or by expression of a transcription factor from rice. Enhanced nitrogen use efficiency is expected to result from expression of a maize transcription factor involved in carbon skeleton production for amino acid synthesis. Sucrose accumulation is expected to be modified with RNAi constructs containing gene fragments from a common crop plant designed to alter sucrose transport, carbohydrate metabolism or osmotic stress tolerance. The efficiency of cellulosic ethanol

[3] The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.

[4] More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030 or at <http://www.ogtr.gov.au/>), and in the Regulator's *Risk Analysis Framework* (OGTR 2007a) at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

production from sugarcane biomass is expected to be improved by expression of bacterial cellulase enzymes, or by silencing of a gene to modify plant cell wall chemical structure.

The purpose of the trial is to evaluate agronomic properties of the GM sugarcane lines grown under field conditions. Promising lines will be selected for crossing under controlled conditions to other GM sugarcane lines or non-GM sugarcane cultivars for possible future commercial development (subject to additional approvals). Expression of the introduced genes will be controlled with a variety of regulatory sequences, with the aim of optimising expression patterns. The GM sugarcane will not be used for human food or animal feed.

BSES proposed a number of controls to restrict the dissemination and persistence of the GM sugarcane lines and their genetic material into the environment. These controls were considered during the evaluation of the application.

Confidential Commercial Information

Some details, including the identities of some of the genes and regulatory sequences, have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

Risk assessment

The risk assessment considered information in the application, relevant previous approvals, current scientific knowledge, and issues relating to risks to human health and safety and the environment raised in submissions received from consultation with a wide range of prescribed experts, agencies and authorities (included in Appendix B of the RARMP) as well as the public (included in Appendix C of the RARMP).

A reference document, *The Biology of the* Saccharum *spp. (Sugarcane)*, was produced to inform the risk assessment process for licence applications involving GM sugarcane plants. The document is available from the OGTR or from the website <http://www.ogtr.gov.au>.

The risk assessment begins with a hazard identification process to consider what harm to the health and safety of people or the environment could arise during this release of GMOs due to gene technology, and how it could happen, in comparison to the non-GM parent organism and in the context of the proposed receiving environment.

Nine events were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

A **risk** is only identified when a hazard is considered to have some chance of causing harm. Events that do not lead to an adverse outcome, or could not reasonably occur, do not represent an identified risk and do not advance any further in the risk assessment process.

The characterisation of the nine events in relation to both the magnitude and probability of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The principal reasons for this include:

- limits on the size and locations of the release proposed by BSES
- suitability of controls proposed by BSES to restrict the dissemination and persistence of the GM sugarcane plants and their genetic material

- limited ability and opportunity for the GM sugarcane line to transfer the introduced genes to other sugarcane plants or other sexually related species
- none of the GM plant materials or products will be used in human food or animal feed
- widespread presence of most of the same or similar proteins and gene sequences encoded by the introduced genes and RNAi constructs in the environment and lack of known toxicity or evidence of harm from them.

Any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM sugarcane into the environment are considered to be **negligible.** Hence, the Regulator considers that the dealings involved in this release **do not pose a significant risk** to either people or the environment.

Risk management

The risk management process builds upon the risk assessment to determine whether measures are required in order to protect people and/or the environment. As none of the nine events characterised in the risk assessment are considered to give rise to an identified risk that requires further assessment, the level of risk is considered to be **negligible**.

The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, conditions have been imposed to restrict the dissemination and persistence of the GMOs and their genetic material in the environment and to limit the release to the size and locations requested by the applicant as these were important considerations in establishing the context for assessing the risks. The context for assessing the risks may change substantially over the 15 year period proposed by the applicant, potentially impacting upon the conclusions of the risk assessment. Therefore, the imposed licence conditions limit the duration of the release to six years.

Licence conditions to manage this limited and controlled release

The Regulator has imposed a number of licence conditions including requirements to:

- limit the release to six years
- surround the field trial sites with one guard row of non-GM sugarcane and a further isolation zone of at least 6 m
- separate GM sugarcane material from non-GM material when propagating seedlings or setts on seedling benches, and clearly identifying GM material
- separate GM from non-GM sugarcane in crossing facilities (glasshouses, pot holding areas, photoperiod glasshouses and crossing shed) and clearly identify GM material
- monitor GM sugarcane in photoperiod facilities for spikelet opening three times weekly and enclose inflorescences in pollen lanterns for controlled crossing, and destroy open spikelets not enclosed in pollen lanterns
- locate the field trial sites at least 50 m away from natural waterways
- harvest and process the GM sugarcane separately from any other sugarcane
- carry out analysis of plant materials only at the BSES stations or in PC2 laboratories
- destroy all plant materials not required for experimentation or propagation
- after cleaning of sites, monitor for and destroy any GM sugarcane that may grow for at least 12 months, and until no volunteers have been detected at the sites for a continuous 6 month period

- transport the GM plant materials in accordance with the Regulator's transportation guidelines
- not allow the GM plant material or products to be used for human food or animal feed.

The Regulator has issued guidelines and policies for the transport, supply and storage of GMOs (*Guidelines for the transport of GMOs, Policy on transport and supply of GMOs*). Licence conditions based on these guidelines and policies have also been imposed to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by other agencies that also regulate GMOs or GM products including Food Standard Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration, National Industrial Chemicals Notification and Assessment Scheme and Australian Quarantine Inspection Service (AQIS)[5].

FSANZ is responsible for human food safety assessment, including GM food. As the trial involves early stage research, the applicant does not intend any material from the GM sugarcane lines proposed for release to be used in human food. Accordingly, the applicant has not applied to FSANZ to evaluate the GM sugarcane lines. FSANZ approval would need to be obtained before they could be sold for use in human food in Australia.

Identification of issues to be addressed for future releases

Additional information has been identified that may be required to assess an application for a large scale or commercial release of these GM sugarcane lines, or to justify a reduction in containment conditions. This would include:

- additional data on the potential allergenicity and toxicity of plant materials from the GM sugarcane lines
- phenotypic characterisation of the GM sugarcane lines, in particular of traits which may contribute to weediness, persistence, altered reproductive capability and ability to disperse in the environment
- molecular characterisation of the GM sugarcane lines
- additional information on potential pollen flow from sugarcane to sexually compatible species.

Suitability of the applicant

The previous Regulator determined, at the commencement of the assessment process for this application, that BSES was suitable to hold a DIR licence under the requirements of section 58 of the Act. The Regulator is satisfied that BSES remains suitable as no relevant convictions have been recorded, and no licences or permits have been cancelled or suspended under laws relating to the health and safety of people or the environment.

[5] More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator (OGTR).
Free call 1800 181 030 or at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

Conclusions of the RARMP

The risk assessment concluded that this proposed limited and controlled release of up to 12,500 GM sugarcane lines on a maximum total area of 21 ha over 15 years in the Queensland local government areas of Moreton Bay, Bundaberg, Mackay, Burdekin and Cairns, poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to restrict the release to the size and locations requested by the applicant as these were important considerations in establishing the context for assessing the risks. The context for assessing the risks may change substantially over the 15 year period proposed by the applicant, potentially impacting upon the conclusions of the risk assessment. Therefore, the imposed licence conditions limit the release to six years, rather than the 15 years proposed by the applicant.

Chapter 1 Risk assessment context

Section 1 Background

1. This chapter describes the parameters within which risks that may be posed to the health and safety of people or the environment by the proposed release are assessed. These include the scope and boundaries for the evaluation process required by the gene technology legislation, details of the intended dealings, the genetically modified organism(s) (GMO(s)) and parent organism(s), previous approvals and releases of the same or similar GMO(s) in Australia or overseas, environmental considerations and relevant agricultural practices. The parameters for the risk assessment context are summarised in Figure 1.

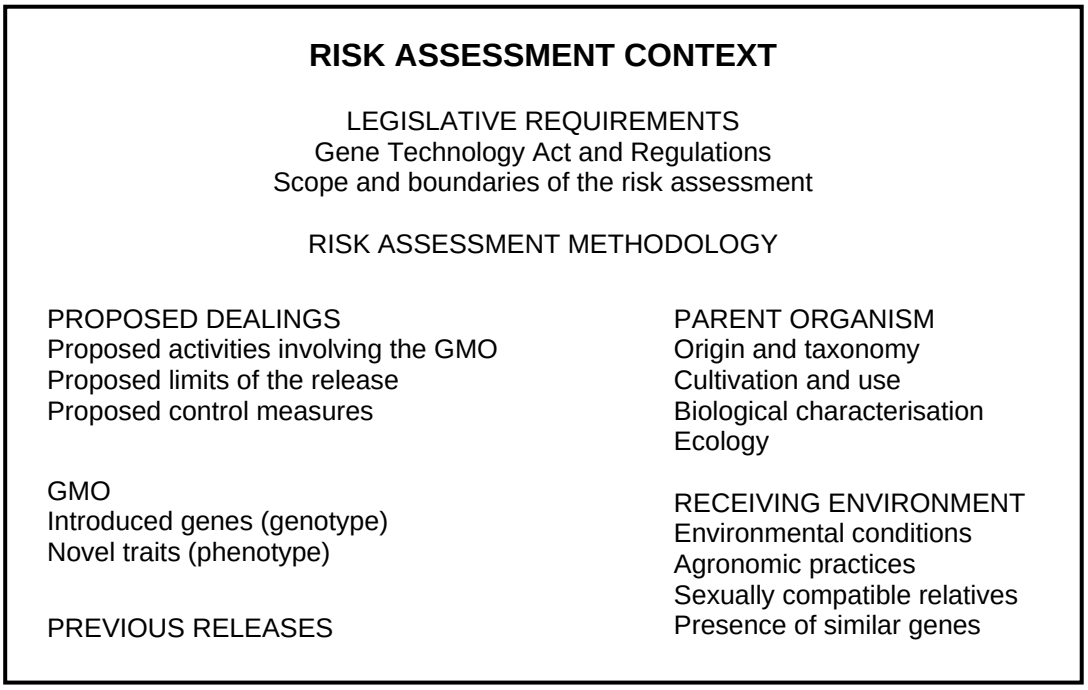

Figure 1. Components of the context considered during the preparation of the risk assessment

2. For this application, establishing the risk assessment context includes consideration of:

- the legislative requirements (Section 2)
- the risk assessment methodology [6]
- the proposed dealings (Section 3)
- the parent organism (Section 4)
- the GMOs, nature and effect of the genetic modifications (Section 5)
- the receiving environment (Section 6)
- previous releases of these or other GMOs relevant to this application (Section 7).

[6] The risk assessment methodology used by the Regulator is outlined in more detail at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/raf-3/$FILE/raffinal3.pdf>

Section 2 The legislative requirements

3. Sections 50, 50A and 51 of the *Gene Technology Act 2000* (the Act) outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and with whom he must consult, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of his decisions on licence applications. In addition, the Gene Technology Regulations 2001 (the Regulations) outline matters the Regulator must consider when preparing a RARMP.

4. In accordance with section 50A of the Act, the previous Regulator considered information provided in the application and was satisfied that its principal purpose is to enable the applicant to conduct experiments. In addition, limits have been proposed on the size, locations and duration of the release and controls have been proposed by the applicant to restrict the dissemination and persistence of the GMOs and their genetic material in the environment. Those limits and controls are such that the previous Regulator considered it appropriate not to seek the advice referred to in subsection 50(3) of the Act. Therefore, this application is considered to be a limited and controlled release and the Regulator has prepared a RARMP for this application.

5. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee (GTTAC), Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, local council(s) where the release is proposed to take place, and the public.

6. Section 52(2)(ba) of the Act requires the Regulator to decide whether one or more of the proposed dealings may pose a 'significant risk' to the health and safety of people or to the environment, which then determines the length of the consultation period as specified in section 52(2)(d). The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix B. Two submissions were received from the public and their consideration is summarised in Appendix C.

Section 3 The proposed dealings

7. BSES Limited (BSES) proposes to release up to 500 sugarcane lines[7] from each of 25 categories[8] of gene constructs (ie a total of up to 12,500 lines) which have been genetically modified (GM) to alter plant growth, enhance drought tolerance, enhance nitrogen use efficiency, alter sucrose accumulation and to improve cellulosic ethanol production from sugarcane biomass, into the environment under limited and controlled conditions.

8. The dealings involved in the proposed intentional release would include:

- conducting experiments with the GMOs
- breeding the GMOs
- propagating, growing, raising or culturing the GMOs
- transporting the GMOs

[7] The term 'line' is used to denote plants derived from a single plant containing a specific genetic modification made by one transformation event.
[8] The applicant originally specified 24 categories of GM sugarcane, with the 25th category being added during the assessment process.

- disposing of the GMOs.

The dealings would also include the possession, supply or use of the GMOs for the purposes of, or in the course of, a dealing mentioned in any of the dealings mentioned above. Those dealings are detailed further throughout the remainder of the current Chapter.

9. Some details of the application concerning the identification of several of the genes and regulatory sequences have been declared Confidential Commercial Information (CCI) under section 185 of the Act. This information was considered during the preparation of the RARMP and was made available to the prescribed expert groups and authorities that were consulted on this application.

3.1 The proposed activities

10. The applicant has stated that the objective of the proposed trial is to evaluate the GM sugarcane plants under field conditions for key changes to agronomic characteristics such as sugar and cane yield and the use of resources such as water and fertiliser. To carry out this objective several rounds of plant production, selection and crossing would be done. The GM sugarcane or products made from it would not be used for human food or animal feed.

11. The GM sugarcane lines proposed for release would be produced in facilities at BSES Indooroopilly (Brisbane) and CSIRO Plant Industry, St Lucia (Brisbane) before being transported to trial sites for planting in the field. In the field, GM sugarcane lines would be assessed for their agronomic potential. Assessment would be carried out in three successive trials (see Figure 2):

- progeny assessment: individual plantlets of all GM lines would be grown in the field and assessed for stalk weight, commercial cane sugar (CCS) yield and other traits at the first harvest. The following ratoon crop would be harvested for planting material for the estimated 10% of lines selected for the next stage of assessment.
- clonal assessment: lines would be planted in long single rows to resemble a commercial planting, and measurements would be taken of crop weight, CCS and other traits (including drought tolerance and nitrogen use efficiency) in two successive harvests. It is estimated that the best 1% of lines would be selected for the next stage of assessment.
- final assessment: lines would be planted in four-row plots and assessed over three harvests for plot weight and CCS. The most promising lines would undergo separate disease screening tests. It is estimated that the best 0.1% of lines would then be maintained in holding plots.

12. Promising GM lines from some of the categories (refer to Table 4 for details) would be selected at the clonal and final assessment stages for crossing, and the resulting progeny would then enter a new round of assessment trials. Crossing would take place over several years, giving rise to several rounds of GM sugarcane undergoing the progression of three assessment trials in later years.

13. All of the work would be undertaken at current or proposed BSES stations (Table 1, Figure 3). Field planting would be done at BSES Burdekin, Central and Southern stations, allowing evaluation of GM plants under a range of different growing environments. BSES also proposes to carry out field plantings at a planned research station (referred to in their application as the proposed GM farm) which would be located in the same geographical region as BSES Burdekin, Central or Southern. At each of these BSES stations the GM sugarcane would be planted in up to ten fields at any one time, and in one seed-raising area. Field planting for the purpose of disease testing would be done at BSES Woodford, where the GMOs would be planted in up to three fields at any one time, and in one seed-raising area.

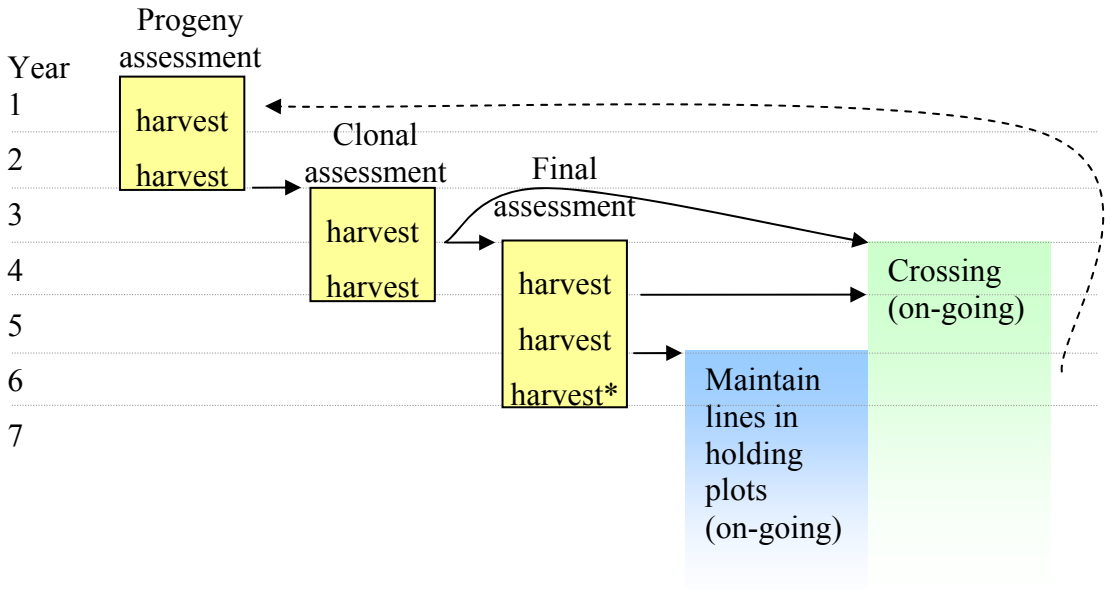

Figure 2. Proposed stages and timing of assessment of GM sugarcane lines

Solid arrows indicate transfer of vegetative material for planting
Dashed arrow indicates transfer of seedlings for commencement of new assessment trials
* the second ratoon of final assessment trials may be harvested for planting material, but yield would not be assessed

Table 1. Proposed localities for release of GM sugarcane

BSES Sugar Experiment Station	Maximum total area (ha)	Maximum number of release sites per season[1]	Local Government Area	Locality
Meringa	0.02	1	Cairns	Meringa, Qld
Burdekin	4	11	Burdekin	Ayr, Qld
Central	4	11	Mackay	Te Kowai, Qld
Southern	4	12	Bundaberg	Bundaberg, Qld
Woodford	4	4	Moreton Bay	Woodford, Qld
Planned station[2]	4	11	Burdekin, Mackay OR Bundaberg	

[1] The different proposed release sites include: at five BSES stations, one nursery area in which GM sugarcane is proposed to be grown in pots; at two BSES stations, one crossing facility site made up of a glasshouse, photoperiod facility and some other areas; and at five BSES stations, up to 10 release sites at which GM sugarcane is proposed to be planted into the field.

[2] The planned station is a facility BSES plans to established in the Burdekin, Mackay or Bundaberg local government area. All other proposed sites are within established BSES Sugar Experiment Stations.

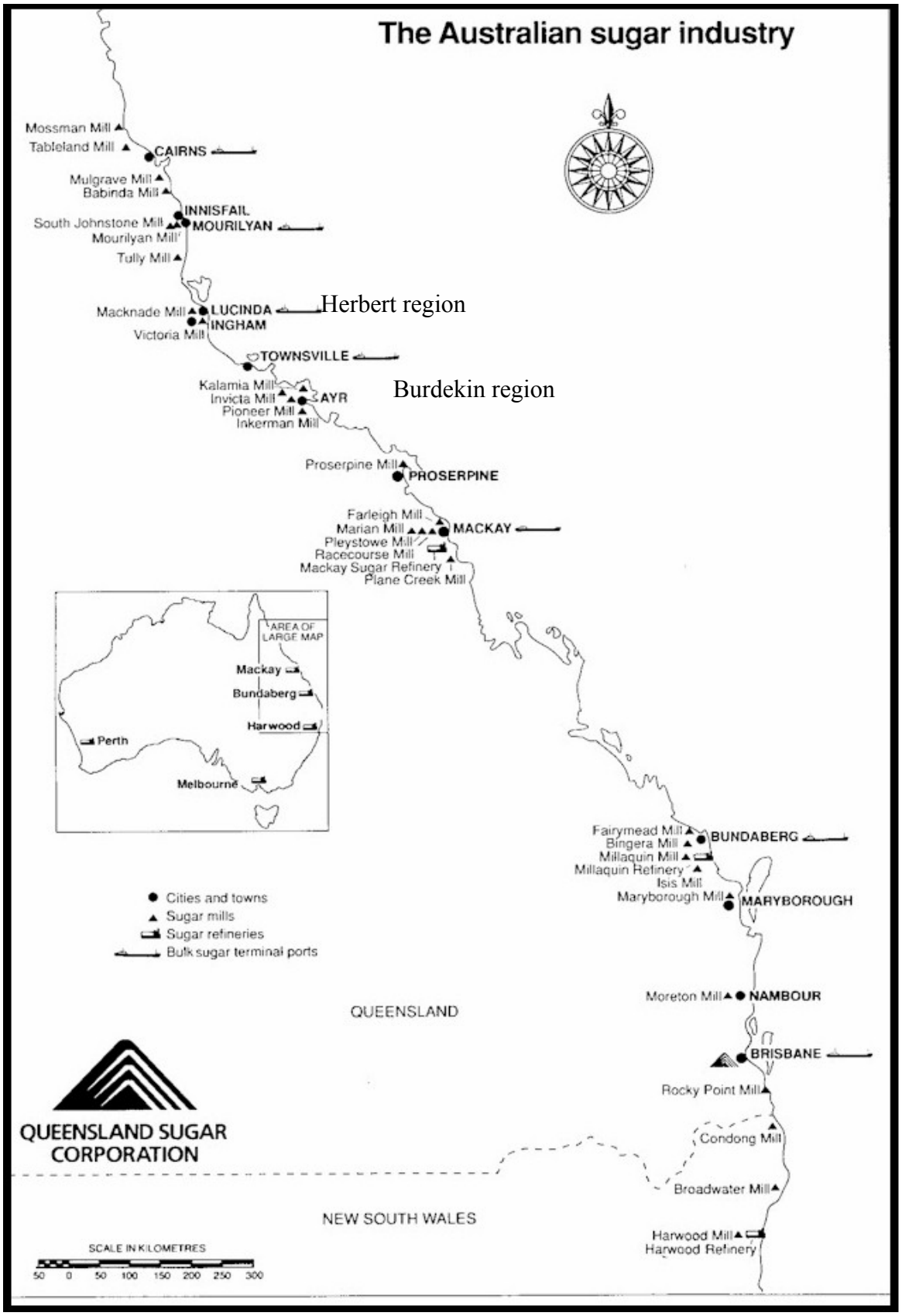

Figure 3. Sugarcane growing areas of Queensland and northern New South Wales. Locations at which the applicant proposes to release GM sugarcane are BSES stations at Woodford (north of Brisbane), Bundaberg (BSES Southern), Mackay (BSES Central), Burdekin (near Ayr), and Meringa (south of Cairns). Map adapted from DIR 095 application.

The facility at BSES Meringa would be used for flower production, crossing, and seed collection and storage. Crossing experiments may also be undertaken at BSES Southern.

3.2 The proposed limits of the dealings (size, locations and duration)

14. The release is proposed to take place at six BSES stations located in the local government areas (LGAs) of Bundaberg Regional Council, Mackay Regional Council, Burdekin Shire Council, Moreton Bay Regional Council and Cairns Regional Council in Queensland on a maximum area of 21 ha over fifteen years from August 2009 to August 2024. Within most of the BSES stations proposed to be used, the release would occur in multiple areas (Table 1). Within the four BSES stations at which general field plantings are proposed (BSES Southern, Central, Burdekin and the planned station), the GM sugarcane lines would be planted in up to 10 field locations at any one time, and also maintained in pots in a nursery area. At BSES Woodford, a nursery area and up to three field planting areas are proposed. At BSES Meringa and BSES Central, an area composed of a glasshouse, a photoperiod facility and some other areas would make up a crossing facility at which the release is proposed to occur.

3.3 The proposed controls to restrict the dissemination and persistence of the GMOs and their genetic material in the environment

15. The applicant has proposed a number of controls to restrict the dissemination and persistence of the GM sugarcane lines and the introduced genetic material in the environment including:

- surrounding the field trial sites by one guard row of non-GM sugarcane and a further isolation zone of at least 6 m
- separating GM sugarcane material from non-GM material when propagating seedlings or setts on seedling benches, and clearly identifying GM material
- monitoring GM sugarcane in photoperiod facilities for spikelet opening and enclosing inflorescences in pollen lanterns prior to spikelet opening
- locating the field trial sites at least 50 m away from natural waterways
- harvesting and processing GM sugarcane from the trial separately from any other sugarcane
- analysing plant materials at the BSES stations or in PC2 laboratories
- destroying all plant materials not required for experimentation or propagation
- after cleaning the sites, monitoring for and destroying any GM sugarcane that may grow for at least 12 months, and until the site is free of volunteers for a continuous 6 month period
- transporting GM plant materials between BSES stations in accordance with the Regulator's transportation guidelines
- not allowing the GM plant material or products to be used for human food or animal feed.

16. These controls, and the limits outlined in Chapter 1, Section 3.2, have been taken into account in establishing the risk assessment context (this chapter), and their suitability for containing the proposed release is evaluated in Chapter 3, Section 4.2.1.

Section 4 The parent organism

17. The parent organism is cultivated sugarcane, *Saccharum* spp. which is an interspecific hybrid of *S. spontaneum* and *S. officinarum*. Sugarcane is exotic to Australia and is

commercially cultivated on the east coast of Australia from northern New South Wales to far north Queensland. The applicant proposes to use any sugarcane cultivar. Further information about the parent organism is contained in a reference document, *The Biology of the* Saccharum *spp. (sugarcane)* that was produced to inform the risk assessment process for licence applications involving GM sugarcane plants (OGTR 2008b). The document is available from the OGTR or from the website <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

Section 5 The GMOs, nature and effect of the genetic modification

5.1 Introduction to the GMOs

18. The applicant proposes to release 25 categories of GM sugarcane with gene sequences from a total of 19 genes of interest, two marker genes and one reporter gene (Table 2). With three exceptions, each category is based upon one full or partial sequence from a gene of interest, which would be combined with different regulatory elements. Two categories would contain two marker genes with and without a reporter gene, and one category would contain a combination of five genes from other categories. Within each category, a group of expression cassettes would be made consisting of the gene(s) of interest in combination with up to five promoters, up to three terminators and up to two targeting sequences (Table 3, Table 4). Within each category, expression cassettes may be cloned into a binary vector for *Agrobacterium*-mediated transformation and up to two different expression vectors for transformation by microprojectile bombardment.

19. The GM sugarcane categories, with the exception of two categories containing only marker and reporter genes, would contain genes that may affect plant growth, drought tolerance, nitrogen use efficiency, sucrose accumulation, and the efficacy of cellulosic ethanol production from sugarcane biomass. Risks associated with dealings with GM sugarcane containing nine of the 19 genes of interest have been previously assessed in the RARMP for DIR 070/2006 (OGTR 2007b), however in the current application these genes are proposed to be combined with a greater variety of promoter sequences, some may be crossed with other lines, and the genes may be introduced into other sugarcane cultivars.

20. Six categories of GM sugarcane would contain partial or complete gene sequences expected to alter plant growth of the GM sugarcane plants, with expected phenotypes including decreased or increased height and decreased or increased tillering. These genes are *PcGA2ox-1* derived from runner bean, *HvGA20ox-1* and *HvGA20ox-2* from barley, *OsTB1* from rice and *ShTB1* from sugarcane.

21. Three categories of GM sugarcane would contain genes expected to confer enhanced drought tolerance. One gene, *OsDREB1A,* is from rice and the other two genes are from a common plant and a common bacterium.

22. One category of GM sugarcane would contain the gene *ZmDof1* from maize, which is expected to confer enhanced nitrogen use efficiency.

23. Seven categories of GM sugarcane would contain partial gene sequences expected to alter sucrose accumulation by modifying sucrose transport, carbohydrate metabolism or osmotic stress tolerance. The partial gene sequences are derived from a common plant species, and are incorporated into constructs designed to decrease expression of homologous genes in sugarcane.

Table 2. The genes used to alter plant growth, improve drought tolerance, improve nitrogen use efficiency, alter sucrose accumulation and improve cellulosic ethanol production from sugarcane biomass in sugarcane

Gene	Genbank Accession	Function of protein	Source	Categories	Intended purpose
*PcGA2ox-1**	AJ132438	Enzyme involved in degradation of active gibberellin	*Phaseolus coccineus* (runner bean)	3	Altered plant growth
*HvGA20ox-1**	AY551428	Enzyme involved in biosynthesis of active gibberellin	*Hordeum vulgare* (barley)	4	Altered plant growth
*HvGA20ox-2**	Unpublished	Enzyme involved in biosynthesis of active gibberellin	*Hordeum vulgare* (barley)	5	Altered plant growth
*OsTB1**	AB088343	Transcription factor regulating axillary bud growth	*Oryza sativa* (rice)	6	Altered plant growth
*ShTB1**	Unpublished	Transcription factor regulating axillary bud growth	*Saccharum* spp. (sugarcane)	7, 8	Altered plant growth
WUE1				9	Improved drought tolerance
WUE2				10	Improved drought tolerance
OsDREB1A[a]	AF300970	Transcription factor potentially regulating drought response	*Oryza sativa* (rice)	11	Improved drought tolerance
*ZmDof1**	D78377	Transcription factor potentially affecting nitrogen use	*Zea mays* (maize)	12	Improved nitrogen use efficiency
SA1	CCI	CCI	CCI	13, 14	Altered sucrose accumulation
SA2	CCI	CCI	CCI	15	Altered sucrose accumulation
SA3	CCI	CCI	CCI	16	Altered sucrose accumulation
SA4	CCI	CCI	CCI	17, 18	Altered sucrose accumulation
SA5	CCI	CCI	CCI	19	Altered sucrose accumulation
SA6	CCI	CCI	CCI	20, 25	Modified plant cell wall chemical structure
SA7	CCI	CCI	CCI	21, 25	Production of cell wall modifying enzymes
SA8	CCI	CCI	CCI	22, 25	Production of cell wall modifying enzymes
SA9	CCI	CCI	CCI	23, 25	Production of cell wall modifying enzymes
SA10	CCI	CCI	CCI	24, 25	Production of cell wall modifying enzymes
*Neomycin phospho-transferase II (nptII)**	AAF65403	Antibiotic resistance	*Escherichia coli*	1-25	Plant selectable marker
*β-lactamase (bla)**	AJ847363	Antibiotic resistance	*Escherichia coli*	1-25	Bacterial selectable marker
*uidA (GUS)**	AY292368	β-glucuronidase	*Escherichia coli*	2	Reporter gene

* Risks associated with dealings with GM sugarcane containing these genes were assessed previously in the RARMP for DIR 070/2006 (OGTR 2007b) , in which *ShTB1* was called *SoTB1*.

[a] The applicant originally specified inclusion of a gene called *OsDREB1* (accession AY196209), which was changed to *OsDREB1A* during the assessment process.

Table 3. The regulatory sequences used in the genetic modification of sugarcane

Regulatory Sequence	Genbank Accession	Function	Source
Ubi1	S94464	Constitutive promoter	*Zea mays* (maize)
P1	CCI	CCI	*Hordeum vulgare* (barley)
P2	CCI	CCI	*Sorghum bicolour* (sorghum)
P3	CCI	CCI	*Arabidopsis thaliana*
P4	CCI	CCI	*Arabidopsis thaliana*
Legumain targeting domain	DQ458784	Vacuolar targeting	*Saccharum* spp. (sugarcane)
Rubisco small subunit targeting domain	X04334	Plastid targeting	*Pisum sativum* (pea)
nos (nopaline synthase)	V00087	Termination region	*A. tumefaciens*
ocs (octapine synthase)	X00493	Termination region	*A. tumefaciens*
tml (tumour morphology large)	X00493	Termination region	*A. tumefaciens*

24. Six categories of GM sugarcane would contain genes, derived from two species of bacteria and a common plant, that are expected to modify the plant cell wall chemical structure or cause sub-cellular accumulation of cell wall degrading enzymes. The aim of these modifications is to increase the efficiency of post-harvest processing of sugarcane biomass for cellulosic ethanol production, through improving recovery of fermentable sugars.

25. One category of GM sugarcane would contain a reporter gene (*uidA*) encoding an enzyme (β-glucuronidase, GUS) that enables visual identification of plant tissues in which this gene is being expressed. The reporter gene was originally derived from the common gut bacterium *Escherichia coli*.

26. All of the GM sugarcane categories would contain an antibiotic resistance selectable marker gene, *neomycin phosphotransferase II* (*nptII*), including one category which would contain no other plant-expressed transgenes. The *nptII* gene was originally derived from the common gut bacterium *E. coli*, and confers resistance to antibiotics such as geneticin and paromomycin on the GM plant. The *nptII* gene would only be used a selective marker during early stages of development of the GM plants in the laboratory.

27. The two categories of GM sugarcane containing only marker and reporter genes are for the purpose of evaluating the effects of the transformation process on the agronomic properties of sugarcane. The agronomic properties of these lines would be used as a baseline against which the applicant could measure how agronomic characteristics were changed in GM lines from other categories.

28. Additionally, expression vectors for biolistic transformation would contain the marker gene *bla* from the bacterium *E. coli*, which confers ampicillin resistance. It is expressed from a bacterial promoter that does not function in plants, so the gene is not expressed in the GM sugarcane plants. The gene would only be used to select bacteria containing the desired genes in the laboratory, prior to the production of the genetically modified plants. In constructs produced for *Agrobacterium*-mediated transformation, the region of DNA to be transferred would not contain *bla*.

29. Short regulatory sequences would be used to control expression of the genes (Table 3). These sequences are derived from plants (including maize, sugarcane and pea), a soil bacterium (*Agrobacterium tumefaciens*) and *E. coli*. Although *A. tumefaciens* is a plant pathogen and *E. coli* is a facultative human pathogen, the regulatory sequences comprise only a small part of their respective total genomes, and are not capable of causing disease.

Table 4. Characteristics of the categories of GM sugarcane

Category	Gene(s)[a]	Construct function	Promoters	Terminators	Targeting sequences	Expected phenotype
1*	*nptII*	Expression	*Ubi1*	*nos*	none	Marker gene expression
2	*nptII* + *uidA*	Expression	*Ubi1*	*nos*	none	Marker and reporter gene expression
3	*PcGA2ox-1*	Expression	Any from Table 3	*nos*	none	Shorter plants
4*	*HvGA20ox-1*	Expression	Any from Table 3	*nos*	none	Taller plants
5*	*HvGA20ox-2*	Expression	Any from Table 3	*nos*	none	Taller plants
6	*OsTB1*	Expression	Any from Table 3	*nos*	none	Decreased no. of tillers
7	*ShTB1*	Expression	Any from Table 3	*nos*	none	Decreased no. of tillers
8	*ShTB1*	RNAi	Any from Table 3	*nos*	none	Increased no. of tillers
9*	*WUE1*	Expression	Any from Table 3	*nos*	Any from Table 3 or none	Improved drought tolerance
10*	*WUE2*	Expression	Any from Table 3	*nos*	none	Improved drought tolerance
11*	*OsDREB1A*	Expression	Any from Table 3	*nos*	none	Improved drought tolerance
12	*ZmDof1*	Expression	Any from Table 3	*nos*	none	Improved nitrogen assimilation
13	*SA1* (fragment 1)	RNAi	Any from Table 3	Any from Table 3	none	Altered sucrose transport
14	*SA1* (fragment 2)	RNAi	Any from Table 3	Any from Table 3	none	Altered sucrose transport
15	*SA2*	RNAi	Any from Table 3	Any from Table 3	none	Increased sugar accumulation
16	*SA3*	RNAi	Any from Table 3	Any from Table 3	none	Altered carbohydrate partitioning
17	*SA4* (fragment 1)	RNAi	*Ubi1*	Any from Table 3	none	Altered carbohydrate partitioning
18[b]	*SA4* (fragment 2)	RNAi	*Ubi1*	Any from Table 3	none	Altered carbohydrate partitioning
19	*SA5*	RNAi	Any from Table 3	Any from Table 3	none	Altered carbohydrate partitioning
20	*SA6*	RNAi	*Ubi1*	*nos*	none	Modified cell wall structure
21	*SA7*	Expression	Any from Table 3	*nos*	Any from Table 3 or none	Accumulation of a cell wall modifying enzyme (no phenotype)
22	*SA8*	Expression	Any from Table 3	*nos*	Any from Table 3 or none	Accumulation of a cell wall modifying enzyme (no phenotype)
23	*SA9*	Expression	Any from Table 3	*nos*	Any from Table 3 or none	Accumulation of a cell wall modifying enzyme (no phenotype)
24	*SA10*	Expression	Any from Table 3	*nos*	Any from Table 3 or none	Accumulation of a cell wall modifying enzyme (no phenotype)
25	*SA6* (RNAi) + *SA7* + *SA8* + *SA9* + *SA10*	Expression and RNAi	Any from Table 3	*nos*	Any from Table 3 or none	Modified cell wall chemical structure and accumulation of cell wall modifying enzymes

[a] The *bla* gene, which is not expressed in plants, would be present in all constructs for biolistic transformation with plasmid vectors. The *nptII* gene would be present in all constructs. All constructs except those noted as RNAi would contain the entire coding region of the selected gene. RNAi constructs would contain partial gene sequences.

[b] This category was not included in the original application, but was added during the assessment process.

* Selected clones from these categories are proposed to be used for crossing to non-GM sugarcane and to other GM lines selected for crossing within this release.

5.2 The introduced genes or RNAi constructs, their encoded proteins and their associated effects

30. In most cases, full gene sequences are proposed to be used for the genetic modifications. The purpose of these modifications is to introduce proteins with new functions into sugarcane, to increase expression of sugarcane genes, or to introduce proteins with similar functions to sugarcane genes which would be expressed in different ways to the endogenous genes.

31. In some instances, partial rather than complete gene sequences are proposed to be used for the genetic modifications, with the aim of decreasing expression of specific endogenous sugarcane genes. The decrease in endogenous gene expression is brought about by a mechanism known as gene silencing or RNA interference (RNAi). RNAi is a plant defence against infecting RNA viruses, and works by using RNA sequences identified by a plant as foreign to recognise matching sequences, which are then destroyed by enzymes (reviewed by Baulcombe 2004). In viral infections, the action of this mechanism means that once a sequence has been recognised as belonging to a virus, any matching sequences belonging to replicating viruses are quickly destroyed.

32. For a transgene to induce RNAi against an endogenous gene, the transcript from the transgene must mimic the structure of the double-stranded RNA viruses which naturally induce RNAi, using sequences from the gene to be silenced (the target gene) (reviewed by Waterhouse & Helliwell 2003). RNAi transgene constructs typically consist of two copies of a fragment of the target gene, arranged to give rise to a single transcript with one forward orientation copy of the target gene sequence followed by one reverse orientation copy. Because the transcript contains identical gene fragments in opposite orientations, they are complementary and naturally base-pair into a double-stranded RNA structure. The double-stranded RNA structure is recognised as being virus-like by the cellular RNAi machinery, which then cuts the transcript into fragments of 21-24 nucleotides. The RNA fragments become sequence guides for enzymes which destroy complementary RNA sequences, including any endogenous transcript with sequence closely matching the transgene. Through this pathway, there is a strong decrease in levels of endogenous transcripts highly similar to the RNAi construct. Because the transcript from the construct is destroyed in this process, no proteins are produced.

5.2.1 Genes expected to alter plant growth

33. Six categories of GM sugarcane expressing sequences from five different genes (*PcGA2ox-1, HvGA20ox-1, HvGA20ox-2, OsTB1* and *ShTB1)* are expected to alter plant growth by affecting characteristics such as internode length and tillering. Risks that may be associated with all of these genes were assessed previously in the RARMP for DIR 070/2006 (OGTR 2007b). Background information on the effects of altered plant growth (plant architecture) on plant productivity has been given in the RARMP for DIR 070/2006 (OGTR 2007b) and will not be discussed here.

34. The genes *PcGA2ox-1* (*P. coccineus Gibberellin 2-oxidase-1*), *HvGA20ox-1* and *HvGA20ox-2* (*H. vulgare Gibberellin 20-oxidase-1* and *-2*) encode enzymes involved in gibberellin (GA) biosynthesis. *PcGA2ox-1* oxidises a biologically active form of GA to an inactive form, resulting in decreased GA activity, and *HvGA20ox-1* and *-2* oxidise an inactive form of GA to an active form, resulting in increased GA activity (Hedden & Kamiya 1997). Biologically active GA generally stimulates internode elongation (reviewed by Raven et al.

1999). These genes would be expressed from a range of promoters in the GM sugarcane lines, resulting in changes to plant height and potentially yield.

35. *OsTB1* (*O. sativa Teosinte branched1*) and *ShTB1*[9] (*Saccharum* hybrid *Teosinte branched1*) encode transcription factors that may affect tillering in the GM sugarcane lines. *OsTB1* and *ShTB1* are homologous to the maize *TB1* gene which controls branching in the maize plant (Doebley et al. 1997; Hubbard et al. 2002). Over-expression of TB1 reduces tillering in GM rice (Takeda et al. 2003; McSteen & Leyser 2005). As in DIR 070/2006, in DIR 095 it is proposed that a complete gene sequences of *ShTB1* and *OsTB1* would be expressed, and a partial gene sequence of *ShTB1* in an RNAi construct would induce silencing of the endogenous sugarcane gene. Expression of the complete gene sequences in GM sugarcane is expected to lead to reduced tillering while RNAi is expected to result in an increase in tillering and reduction in height.

5.2.2 Genes expected to improve drought tolerance

36. Three categories of GM sugarcane expressing three different genes (*WUE1*, *WUE2* and *OsDREB1A*) are expected to have enhanced drought tolerance. Background information on the effects of enhanced drought tolerance on plant productivity has been given in the RARMP for DIR 070/2006 (OGTR 2007b) and will not be discussed here. Assessment of the risks associated with dealings with GM sugarcane under DIR 070/2006 involved different genes to those expected to confer enhanced drought tolerance in the current application. The specific identities of *WUE1* and *WUE2* and the phenotypes resulting from their expression have been declared CCI and are not discussed further in this Section.

37. The gene *OsDREB1A* (*O. sativa Dehydration-responsive element-binding protein 1A*) from rice encodes a transcription factor belonging to the 139-member ERF family. This family is divided based upon amino acid sequence of the DNA-binding AP2 domain: there are two sub-families, the CBF/DREB subfamily (to which *OsDREB1A* belongs) and the ERF subfamily. The CBF/DREB subfamily is comprised of four major subgroups, with *OsDREB1A* belonging to subgroup III (Nakano et al. 2006). These divisions are thought to reflect changes in DNA-binding specificity which may have biological significance. While most members of this large family are unstudied, known functions are diverse. Members of CBF/DREB group III are known to have roles in abiotic stress responses. Several, including OsDREB1A, have been shown to bind to a DNA sequence known as the dehydration-responsive element (DRE), a promoter element common to genes up-regulated in response to drought, high-salt and cold stresses (Dubouzet et al. 2003). Through binding to DREs, DREB proteins can mediate broad transcriptional responses to dehydration stress. Over-expression of some DREB genes, including *OsDREB1A*, has been shown to result in increased drought tolerance (reviewed by Nakashima et al. 2009). For example, in *Arabidopsis thaliana*, strong constitutive expression of *A. thaliana* DREB1A was found to confer drought tolerance and cause severe growth retardation, while expression of DREB1A from a stress-responsive promoter minimised negative effects on growth while still conferring stress tolerance (Kasuga et al. 1999).

5.2.3 Genes expected to improve nitrogen use efficiency

38. Background information on the effects of enhanced nitrogen use efficiency on plant productivity has been given in the RARMP for DIR 070/2006 (OGTR 2007b) and will not be discussed here. The gene expected to confer enhanced nitrogen use efficiency on GM

[9] *ShTB1* was referred to as *SoTB1* in the RARMP for DIR 070/2006

sugarcane lines assessed under DIR 070/2006 was the same gene as is included in the current application.

39. One category of GM sugarcane expressing a DNA-binding with one finger (Dof) transcription factor is expected to have enhanced nitrogen use efficiency. Dof transcription factors are specific to plants and have a wide range of functions which they carry out by binding to promoter elements and enhancing expression of target genes (reviewed by Yanagisawa 2002). They are also known to bind to other proteins, including bZIP transcription factors (reviewed by Yanagisawa 2004). *ZmDof1* (*Z. mays Dof1*) is thought to regulate multiple light-responsive genes involved in synthesis of organic acids from fixed carbon, a process which gives rise to the carbon skeleton from which amino acids are synthesised following nitrogen assimilation (reviewed by Yanagisawa 2004). A study in *A. thaliana* showed that constitutive expression of *ZmDof1* resulted in up-regulation of genes involved in carbon skeleton production (Yanagisawa et al. 2004). In these plants an increase in amino acid content was observed, along with enhanced nitrogen assimilation under normal conditions and increased growth under low nitrogen conditions. Expression of *ZmDof1* in GM sugarcane may result in similar improvements to nitrogen assimilation, which may improve plant growth when nitrogen fertiliser inputs are reduced.

5.2.4 Genes expected to alter sucrose accumulation

40. Sugar is manufactured during photosynthesis in leaves, mainly in the form of sucrose, and is transported in the phloem to other parts of the plant for growth and, in sugarcane, to the stem for storage. Large amounts of sucrose are deposited in the sugarcane stem in both the vacuoles of storage parenchyma cells and the apoplast (cell wall and intercellular spaces) surrounding these cells (reviewed by Braun & Slewinski 2009). Sugarcane is able to store significant amounts of sucrose and a mature stem can accumulate up to approximately 17% of its fresh weight as sucrose (Bull & Glasziou 1963).

41. Increasing the sucrose content of sugarcane is a major objective of most sugarcane improvement programmes. Grof and Campbell (2001) identified the four rate-limiting steps of sucrose accumulation as the leaf reactions which produce sucrose, the rate of phloem loading for transport to the stem, the rate of transport into storage parenchyma and the rate of sucrose remobilisation for vegetative growth. Genes involved in aspects of these processes have been identified, including genes encoding proteins involved in sucrose synthesis, cleavage and transport, and carbon partitioning and storage (for example see Lakshmanan et al. 2005; Moore 2005; Casu et al. 2005). Approaches to altering rate limiting steps to increase accumulation of sucrose and other sugars include the use of genetic modification. For example, expression of a bacterial sucrose isomerase in storage parenchyma cells has been shown to result in conversion of sucrose to an isomer which is not metabolised by the plant, leading to strong increases in sugar accumulation (Wu & Birch 2007). It is thought that sugarcane is capable of accumulating sucrose to more than 25% of fresh weight, should rate-limiting steps be overcome (reviewed by Grof & Campbell 2001)

42. In the current application seven categories of GM sugarcane contain modifications which may lead to altered sucrose accumulation, all accomplished by use of RNAi constructs designed to decrease expression of sugarcane genes. The specific identities of these genes and the phenotypes resulting from their silencing have been declared CCI and are not discussed further in this Section.

5.2.5 Genes expected to improve cellulosic ethanol production from sugarcane biomass

43. Fermentation of sugars produces ethanol, a biofuel of value as a substitute for petroleum. The vast majority of current ethanol production from plant crops comes from the

fermentation of starch and sugar, sourced predominantly from maize grain and sugarcane, respectively. Although cellulose is the most abundant plant carbohydrate available, it is not used for ethanol production because is significantly more expensive to produce ethanol from cellulose than starch or sugar. A major focus of current biofuel research is reducing costs and improving methods of cellulosic ethanol production, so as to enable production from a wider range of feedstock crops and avoid the use of food crops (reviewed by Sticklen 2008). If cellulosic ethanol production were to become feasible, a broad range of waste biomass remaining after crop harvest could be used to produce ethanol, including sugarcane stems from which cane juice has been extracted.

44. The major impediment to commercial cellulosic ethanol production is the expense of current production methods, in particular, the cell-wall hydrolysis enzymes needed for conversion of cellulose (a complex carbohydrate) to fermentable sugars such as glucose. Such enzymes are typically produced from fungi and bacteria in bioreactors. Current research into the production of cellulases *in planta* aims to decrease or eliminate the need to add enzymes during biomass processing (reviewed by Sticklen 2008). The current application includes five categories of GM sugarcane expressing four bacterial cellulase genes, either individually (categories 21-24, Table 4) or in combination (category 25, Table 4). Also included is a gene fragment derived from a common crop plant for modification of plant cell wall structure, also for the aim of improving cellulosic ethanol production from sugarcane biomass (individually in category 20 and combined with bacterial cellulase enzymes in category 25). The specific identities of these genes and the phenotypes expected to result from their expression have been declared CCI and are not discussed further in this Section.

5.2.6 Toxicity/allergenicity of the end products associated with the introduced genes or RNAi constructs

45. Risks associated with dealings with GM sugarcane containing the genes expected to give rise to altered plant growth and enhanced nitrogen use efficiency were previously assessed in the RARMP for DIR 070/2006, which concluded that there was no evidence in published scientific literature to suggest that they may be toxic or allergenic when expressed in GM sugarcane (OGTR 2007b). These genes were isolated from runner bean, barley, rice and sugarcane and homologues of all of the encoded proteins occur naturally in a range of organisms, including plants widely consumed by people and animals. On this basis humans and other organisms have a long history of exposure to these genes and their expressed proteins.

46. The *WUE1* and *OsDREB1A* genes, which are expected to improve drought tolerance, were isolated from a common food plant and rice, respectively (Table 2), and as such humans and other organisms have a long history of exposure to these genes and their expressed proteins. *WUE2* is derived from a soil bacterium, and its product is not known to be toxic or allergenic.

47. The sequences expected to improve cellulosic ethanol production from sugarcane biomass through accumulation of cellulolytic enzymes are derived from two non-pathogenic bacteria.

48. All sequences expected to alter sucrose accumulation in the GM sugarcane lines are expressed from RNAi constructs designed to reduce the levels of endogenous sugarcane transcripts, and no new proteins are expected to be produced. The secondary effects of the RNAi constructs are expected to be modifications to sucrose transport, accumulation and metabolism. It is not anticipated that such changes would alter the allergenicity or toxicity of sugarcane on the basis that sucrose is not toxic unless consumed in large quantities. The oral dose of sucrose required to kill 50% of tested rats is 29 grams per kilogram of body weight (Boyd et al. 1965).

49. A comprehensive search of the scientific literature yielded no information to suggest that any of the encoded proteins are toxic or allergenic to people, or toxic to other organisms.

50. No studies on the toxicity or allergenicity of the GM sugarcane lines and their products have been undertaken to date as the proposed trial is at an early stage. Such studies would have to be conducted if approval was sought for the GMOs or their products to be considered for human consumption in Australia.

*5.2.7 The plant antibiotic resistance marker gene (*nptII*) and the encoded protein*

51. The *nptII* gene is used extensively as a selectable marker in the production of GM plants (Miki & McHugh 2004). As discussed in previous DIR RARMPs, and in more detail in the RARMPs for DIR 070/2006 and DIR 074/2007 (available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir070-2006> and <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir074-2007> or by contacting the OGTR), regulatory agencies in Australia and in other countries have assessed the use of the *nptII* gene in GMOs as not posing a risk to human or animal health or to the environment. The most recent international evaluation of *nptII* in terms of human safety was by the European Food Safety Authority, which concluded that the use of the *nptII* gene as a selectable marker in GM plants (and derived food or feed) does not pose a risk to human or animal health or to the environment (EFSA 2007).

*5.2.8 The bacterial antibiotic resistance marker gene (*bla*) and the encoded protein*

52. Some of the GM sugarcane lines in the proposed release will contain the *β-lactamase* (*bla,* also known as *amp*) antibiotic resistance marker gene. The *bla* gene is derived from *E. coli* (Spanu et al. 2002) and encodes the β-lactamase enzyme, which confers ampicillin resistance.

53. The β-lactamase enzyme is widespread in the environment and in food. Naturally occurring ampicillin-resistant microorganisms have been found in mammalian digestive systems (Spanu et al. 2002). The *bla* gene was originally isolated from antibiotic resistant strains of *E. coli* found in hospital patients.

54. A number of GM crops containing the *bla* gene have been approved for limited and controlled release in Australia (eg papaya in DIR 026/2002, sugarcane in DIRs 051/2004, 070/2006 and 078/2007, and rice in DIR 052/2004). No adverse effects on humans, animals or the environment have been reported from these releases.

*5.2.9 Reporter gene (*uidA*) and its encoded protein (GUS)*

55. The *uidA* gene encodes the enzyme β-glucuronidase (GUS), which is derived from the common gut bacterium *E. coli*. The GUS protein is a monomer with a molecular weight of 68 kDa, and the GUS enzyme is active as a tetramer. GUS catalyses the hydrolysis of β-glucuronides and, less efficiently, some β-galacturonides. *E. coli* lives in the digestive tract of vertebrates, including humans (Jefferson et al. 1986), and the GUS enzyme enables it to metabolise β-glucuronides as a main source of carbon and energy.

56. The *uidA* gene is the most widely used reporter gene in GM plants (Miki & McHugh 2004) as it allows GM tissues to be identified using a simple visual assay. A number of GM crops containing the *uidA* gene have been approved for limited and controlled release in Australia (eg papaya in DIR 026/2002, pineapple in DIR 028/2002, grapevine in DIR 031/2002, rice in DIR 052/2004, sugarcane in DIR 070/2006 and cotton in DIR 074/2007). No adverse effects on humans, animals or the environment have been reported from these releases. The US EPA does not consider the GUS protein to be toxic and has approved its exemption from the requirements to establish tolerance levels (EPA 2001). Safety

assessments by FSANZ of GM foods containing the *uidA* gene have concluded that its presence poses a negligible risk to human health and safety (FSANZ 2002; for example see FSANZ 2003).

5.3 The regulatory sequences

5.3.1 Regulatory sequences for expression of the introduced genes or RNAi constructs

57. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription. Five promoters are proposed to be used in the constructs (Table 3). The maize *Ubiquitin1* (*Ubi1*) promoter is a constitutive promoter that is widely used in plant genetic modification (Christensen et al. 1992). Four other promoters would be used to obtain greater specificity of expression, and in each case the promoter sequence would be followed by the maize *ubiquitin1* intron to enhance gene expression. The specific identities of these genes have been declared CCI and are not discussed further in this Section.

58. Also required for gene expression in plants is an mRNA termination region, including a polyadenylation signal. The mRNA termination regions used in the GM sugarcane lines would be derived from three *A. tumefaciens* genes: *nopaline synthase* (*nos*), *octopine synthase* (*ocs*) and *tumour morphology large* (*tml*). Each of these sequences has been used in a wide variety constructs for plant genetic modification, *nos* being very commonly used (Reiting et al. 2007). Although *A. tumefaciens* is a plant pathogen, the regulatory sequences comprise only a small part of its total genome, and are not capable of causing disease.

59. In RNAi constructs, separation of the inverted-repeat arms with a spliceable intron has been shown to increase the effectiveness of silencing (Smith et al. 2002). RNAi constructs for use in GM sugarcane are to be produced in the vector pStarling, in which fragments of the silencing target gene are cloned on either side of a barley *Cereal cyst nematode resistance* (*cre*) gene intron.

5.3.2 Regulatory sequences for the expression of the nptII, uidA *and* bla *genes*

60. All of the GM sugarcane lines in the proposed release are to include the *nptII* plant selectable marker. In lines generated by biolistic transformation, the construct containing the gene(s) of interest would be co-bombarded with a plasmid containing *nptII* under the control of the *Z. mays Ubi1* promoter and the *A. tumefaciens nos* terminator. In lines generated by *Agrobacterium*-mediated transformation, the *nptII* gene under the control of the same regulatory elements would be incorporated in the transfer DNA (T-DNA) region of the binary vector containing the gene of interest (see below).

61. The *uidA* marker gene will also be under the control of the *Z. mays Ubi1* promoter and the *A. tumefaciens nos* terminator.

62. The *bla* gene in the GM sugarcane lines will be under the control of its own bacterial promoter and terminator from *E. coli* and therefore would not be expressed in the GM sugarcane plants. The gene would be used in the laboratory prior to the production of the GM sugarcane lines.

5.3.3 Sequences for subcellular targeting of products of the introduced genes

63. The applicant proposes to use targeting sequences in some of the constructs (Table 3) to direct protein products into specific cellular compartments. Targeting sequences (also known as transit peptides) are amino acid motifs located at one end of a protein which contain information for protein targeting and transport (Buchanan et al. 2000). Specific targeting motifs have been found to target proteins to various organelles. This is a general approach used to increase protein production (Benchabane et al. 2008). For example, subcellular

targeting has been successfully used to obtain high levels of expression of methylmercury lyase in plants (Bizily et al. 2003). Targeting the methylmercury lyase to either the endoplasmic reticulum or the cell wall resulted in GM plants with a much higher resistance to organic mercury than those GM plants in which the enzyme was expressed without a targeting sequence. The two targeting sequences to be used are the sugarcane legumain vacuole-targeting sequence and the *Pisum sativum* ribulose-1'5'-bisphosphate carboxylase/oxygenase (Rubisco) small subunit chloroplast-targeting domain. Depending upon the targeting sequence, the applicant proposes they would be fused to either the 5' or 3' end of the gene of interest (each targeting sequence would not be trialled at both ends of the gene of interest).

64. Jackson et al. (2007) used a bioinformatic approach to identify vacuolar proteins of sugarcane, and identified a legumain gene containing a vacuolar targeting motif. Legumains are asparagine-specific cysteine proteinases that, in plants, function in vacuolar compartments to process and degrade proteins (Muntz 2007). Legumains are also known as vacuole processing enzymes (VPEs). *Saccharum officinarum* VPE-1 is a legumain homologue with a conserved motif within its N-terminal propeptide that directs the protein into the lytic vacuole. The minimal vacuolar targeting motif, consisting of five amino acids, has been shown to direct GFP to the lytic vacuole of sugarcane when expressed as a translational fusion at the N-terminus of GFP (Jackson et al. 2007). The primary function of lytic vacuoles is amino acid recycling (Muntz 2007), and so Jackson et al. (2007) speculated that protein modifications may be necessary for avoiding degradation of proteins of interest targeted to the lytic vacuole. Vacuolar targeting has previously been successfully used to accumulate significant amounts of various transgene products (Benchabane et al. 2008).

65. The Rubisco small subunit (RbcS) is a nuclear-encoded protein which is localised to the chloroplast. Chloroplasts are a type of plastid, a group of organelles specialised for photosynthesis, storage and biosynthesis. RbcS is typical of plastid targeted proteins in that its targeting motif is an N-terminal extension (Ko et al. 2006). The RbcS targeting motif from a variety of plants has been used in a wide variety of applications to target transgene products to plastids. The *P. sativum* RbcS plastid targeting sequence has previously been successfully used in sugarcane to target three enzymes for polyhydroxybutyrate production to plastids (Petrasovits et al. 2007).

5.4 Method of genetic modification

66. The applicant proposes to use biolistic and *Agrobacterium*-mediated transformation methods to generate the GM sugarcane lines in the proposed release.

67. The biolistic transformation methods used would be based upon published methods (Bower & Birch 1992; Bower et al. 1996). Briefly, sugarcane embryogenic tissue is bombarded with tungsten particles coated with the DNA to be introduced. In the current application, this consists of a marker plasmid carrying *nptII* and a plasmid carrying an expression cassette for the sequences of interest. The applicant plans to perform biolistic transformation using either circular plasmid DNA or linearised plasmid DNA from which backbone sequences (including *bla*) have been removed. After bombardment, transformed sugarcane cells are tissue cultured under selection (on the basis of geneticin resistance conferred by the *nptII* gene), and regenerated into plantlets. The biolistic transformation method has been extensively used and is discussed in previous RARMPs including DIR 051/2004 and DIR 077/2007 (OGTR 2005; OGTR 2008a).

68. The applicant also proposes to generate GM sugarcane lines using *Agrobacterium*-mediated transformation. *A. tumefaciens* is a common gram-negative soil bacterium that causes crown gall disease in a wide variety of plants (Van Larebeke et al. 1974). Plants can be genetically modified by the transfer of DNA (transfer-DNA or T-DNA, located between specific border sequences on a resident plasmid) from *A. tumefaciens* through the mediation

of genes from the virulence region of tumour-inducing plasmids. In the current application, expression cassettes carrying the sequences of interest would be introduced into the T-DNA region of binary vectors also carrying an *nptII* marker gene. Briefly, sugarcane callus would be treated with a culture of *A. tumefaciens* carrying a binary vector, then transformed cells would be regenerated to plantlets on media containing the selective agents paramomycin (resistance to which is conferred by *nptII*) and timentin (which suppresses *A. tumefaciens*). The applicant plans to PCR test DNA extracted from leaves of regenerated plantlets for the presence of *Agrobacterium*, to ensure it does not persist in plantlets to be transferred to the field.

69. There has been concern in a recent publication that transfer of *A. tumefaciens* chromosomal DNA to the plant host may, in 0.4% of cases, accompany the integration of *A. tumefaciens* DNA flanked by the T-DNA borders (Ulker et al. 2008). However, the likelihood of *A. tumefaciens* chromosomal DNA having an influence on any resulting GM plants is regarded as small given the low likelihood of plants possessing the DNA segments necessary for expression of the *A. tumefaciens* genes.

70. Each GM sugarcane line generated from an independent biolistic or *Agrobacterium*-mediated transformation event is expected to have the transgenes located at different sites in the sugarcane genome. Both biolistic and *Agrobacterium*-mediated transformation have been widely used in Australia and overseas for introducing new genes into plants and are not known to cause any adverse effects on human health and safety or the environment.

5.5 Characterisation of the GMOs

5.5.1 Stability and molecular characterisation

71. The applicant states that all genes to be introduced into sugarcane have been sequenced. Molecular characterisation of the GM sugarcane lines has not been carried out and stability of the genetic modifications is also unknown, as the project is in a very early stage. The applicant proposes to plant individual, uncharacterised, primary transformant plantlets to the field following screening to confirm the GM nature of plantlets.

72. Previous studies have established that genes integrated into the sugarcane genome by particle bombardment are stable (Umbeck et al. 1989; Perlak et al. 1990; Harrison et al. 2001). To demonstrate the inheritance and expression of the introduced genes, Harrison et al. (2001) crossed GM sugarcane lines with a non-GM sugarcane line. The proportion of progeny expressing the introduced gene was 1:1 indicating that the introduced genes were inherited in the normal Mendelian fashion.

73. Genes introduced into other GM sugarcane lines have also been found to be stably inherited and expressed in clones propagated via stem cuttings (Hansom et al. 1999). Sugarcane is normally propagated asexually by stem cuttings.

5.5.2 Characterisation of the phenotypes of the GM sugarcane lines

74. The proposed trial is for the purpose of characterising the phenotypes of the GM sugarcane lines in the field, and they have not undergone any prior characterisation. The applicant states that this is necessary because of limited PC2 glasshouse space and variable results of glasshouse trials.

75. Licence DIR 070/2006 authorised BSES to release GM sugarcane lines carrying some of the same genes and partial gene sequences as are described in the current application (Table 2, Table 4 categories 1-8 and 12). In DIR 070/2006 all genes and partial gene sequences were under the control of the constitutive maize *Ubi1* promoter. In the current application, with the exception of *nptII* and *uidA*, all of the sequences common to DIR 070/2006 are proposed to be expressed from the maize *Ubi1* promoter and four other

promoter sequences thought to give more specific expression. Bearing this in mind, phenotypes reported for GM sugarcane lines released under DIR 070/2006, discussed below, are likely to bear similarities to those expected for related GM sugarcane lines in the current application. Phenotypes discussed below are based upon data provided by the applicant, which generally showed that a range of phenotypes occur for each construct. They are based upon comparisons to NPTII-expressing GM control lines, and are based upon the median of between two and 16 independent lines, each measured at least twice, from plants grown at BSES Woodford in one season. Comparison of the GM sugarcane lines to NPTII control lines is used instead of comparison to non-GM sugarcane as all plants which have been transformed show substantial decreases in weight, stem diameter and cane yield. In order to determine the effect of each specific genetic modification on sugarcane phenotype, the GM lines must be compared to other plants which have undergone transformation and regeneration in tissue culture.

GM sugarcane lines with modified plant growth

76. Lines expressing *HvGA20ox-1* and *-2* from the *Ubi1* promoter showed a median increase in plant height of approximately 50% and a median decrease in stalk number of approximately 40-50%, and no large changes in stalk diameter. In the *HvGA20ox-1* and *-2* lines these changes resulted in cane yield being reduced by 25% and 50%, respectively, and sugar yield being reduced by 10% and 25%, respectively. The range of phenotypes observed for *HvGA20ox-2* lines was particularly variable. The applicant has stated that expression of both *HvGA20ox* genes was only expected to affect plant height, with the effects on tillering being unexpected. The applicant has also stated that it is possible that buds on the stem nodes of these lines may possibly mature more quickly.

77. Lines expressing *PcGA2-ox* from the *Ubi1* promoter generally display opposite phenotypes to the *HvGA20ox* lines: height was reduced by approximately 35%, stalk number was increased by approximately 30%, and stem diameter showed no large changes. As a result of these changes reductions in cane yield of approximately 45% and sugar yield of approximately 25% were observed. Similarly to the *HvGA20ox-2* lines, phenotypic variability between lines was high. The applicant states that plants expressing *PcGA2ox* may be more resistant to lodging (falling over, typically due to heavy rain or wind).

78. Lines expressing *OsTB1* and *ShTB1* from the *Ubi1* promoter showed little difference to control plants in height, stalk diameter, cane yield and sugar yield. *OsTB1* lines showed an approximately 25% decrease in stalk number. In lines expressing RNAi constructs against *ShTB1* from the *Ubi1* promoter there was little effect upon plant height, an increase in median stalk number of approximately 20% and a small decrease in stem diameter of approximately 10%, with very high variability being observed between lines. These changes resulted in an approximately 35% reduction in cane yield, with sugar yield not being substantially changed.

GM sugarcane lines with enhanced nitrogen use efficiency

79. In lines expressing *ZmDof1* from the *Ubi1* promoter plant height was increased by approximately 20%, while small decreases in stalk number and stalk diameter were observed. As a result of these changes an approximately 20% increase in cane yield was observed, while CCS was unchanged from the control lines.

Section 6 The receiving environment

80. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes the geographic regions where the release would occur and any relevant biotic/abiotic properties of these locations; the intended agronomic practices, including those that may be altered in relation to normal practices; other

relevant GMOs already released; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed release (OGTR 2007a).

81. The proposed dealings involve planting GM sugarcane in the field within five BSES Sugar Experiment Stations (BSES Woodford, Southern, Central and Burdekin and at the planned BSES station), and cultivating GM sugarcane for crossing at a sixth BSES station (BSES Meringa, Table 1). BSES Southern could also be used for crossing. All BSES stations are in agricultural areas of Queensland on the outskirts of rural towns. Sugarcane is grown in the immediate vicinity of each of the established BSES stations proposed to be used for the trial, on both commercial sugarcane farms and within the experiment stations, except at BSES Woodford which is not close to commercial sugarcane farms. All BSES stations can only be accessed via private roads.

6.1 Relevant abiotic factors

82. The abiotic factors relevant to the growth and distribution of commercial sugarcane in Australia are discussed in *The Biology of the* Saccharum *spp. (sugarcane)* (OGTR 2008b).

83. The location of one of the BSES stations proposed to be used for the trial is yet to be determined, and the applicant states that it would be located in the Burdekin, Mackay or Bundaberg LGAs. As other BSES stations in the application are located in each of these LGAs, the general features of the receiving environment at the planned station have been taken into consideration.

84. The release is proposed to take place in the Queensland LGAs of Burdekin, Moreton Bay, Cairns, Bundaberg and Mackay. The Cairns and Burdekin regions have a tropical climatic type and the Mackay, Bundaberg and Moreton Bay regions have a sub-tropical climatic type (as defined by the Koeppen Classification system used by the Australian Bureau of Meteorology). The rainfall and temperature statistics for the proposed release sites or nearest locations are given in Table 5. With the exception of BSES Woodford, the proposed field locations are on flat arable land at minimal risk of flooding (information supplied by the applicant) and located at least 50 m from the nearest waterway, with the exception of one part of the crossing facility at BSES Meringa. BSES Woodford is located on a hilly site at which the high parts of the site proposed to be used for the trial have no history of flooding, and the GM sugarcane trial would be located at least 50 m from the nearest waterway. To date no incidents of dispersal of sugarcane plant material as a result of cyclones or storms have been reported.

Table 5. Monthly temperature and rainfall statistics for proposed release sites*

Site	Nearest weather station	Mean max temp (°C) Summer	Mean min temp (°C) Summer	Mean max temp (°C) Winter	Mean min temp (°C) Winter	Mean monthly rainfall (mm) Summer	Mean monthly rainfall (mm) Winter
BSES Woodford	Crohamhurst	28.6	18.4	20.1	7.6	249	80
BSES Southern	Bundaberg post office	30.1	21.0	22.5	10.6	170	51
BSES Central	Te Kowai	30.8	21.6	23.8	10.8	294	43
BSES Burdekin	Burdekin Shire Council	32.1	22.1	25.8	12.5	213	21
BSES Meringa	Cairns airport	31.3	23.6	26.0	17.4	342	35

*data taken from the Australian Bureau of Meteorology website (http://www.bom.gov.au/climate/averages/) Temperature and rainfall data are an average of 66 to 118 years of records. Summer entries are averages of monthly data from December to February, and Winter entries are averages of monthly data from June to August.

6.2 Relevant biotic factors

85. The biotic factors pertaining to the growth and distribution of commercial sugarcane are discussed in *The Biology of the* Saccharum *spp. (sugarcane)* (OGTR 2008b), and of relevance to this release are the following points:

- The proposed release sites are on research stations or farms surrounded by commercial sugarcane growing fields.
- GM sugarcane lines released under DIR 070/2006 and DIR 078/2007 are being grown at BSES Woodford, Southern, Central, and Meringa research facilities at which the current release is proposed to take place. Traits of these other GM sugarcane lines are altered sugar production, enhanced nitrogen use efficiency, enhanced water use efficiency and altered plant architecture.
- Invertebrates, vertebrates and microorganisms are expected to be exposed to the introduced genes or RNAi constructs, their encoded proteins and end products. Fauna including rats and feral pigs may have access to the sites. To date there is no record of dispersal of sugarcane plant material by these vertebrates.

6.3 Relevant agricultural practices

86. The size, locations and duration of the proposed limited and controlled release of the GM sugarcane lines are outlined in Section 3.2 of this Chapter.

87. In Queensland commercial sugarcane is planted from autumn to spring and harvested after 12 –18 months. In the proposed release the GM sugarcane is to be grown in the field in 12 month planting seasons.

88. The proposed release consists of three rounds of assessment trials in which GM sugarcane lines are to be grown in the field and assessed for agronomic performance (Figure 2) in addition to disease testing, crossing, and maintenance of GM sugarcane lines in holding plots. Each trial consists of assessing agronomic characteristics of a single planting of sugarcane from planting to harvest at 12 months and at successive harvests of one or more ratoon crops at 12 month intervals. With successive trials a decreasing number of clones are to be selected and grown on increasingly larger scales under conditions more closely resembling commercial plantings. The most promising lines are to undergo disease screening and are proposed to be used in crosses from which progeny would enter new rounds of assessment trials.

89. The applicant proposes to carry out planting and harvest by hand or using machinery. For the purpose of minimising the spread of diseases, planting and harvest machinery used on BSES stations is routinely cleaned between uses (information supplied by the applicant). The applicant has proposed measures to distinguish field plantings of GM sugarcane from non-GM sugarcane so as to reduce the possibility of inadvertent harvesting of GM material, including separation of GM from non-GM sugarcane with a guard row of non-GM sugarcane and a 6 m isolation zone, marking GM plantings with signs and star pickets and implementing staff management procedures to inform harvest staff of GM sugarcane locations.

90. The initial GM sugarcane lines would be evaluated in progeny assessment trials in the first two years of the release. The applicant intends to transport tissue-cultured GM sugarcane plantlets which would be produced in PC2 facilities in Brisbane to four BSES stations, BSES Southern, Central, Burdekin and the planned station. At each station the plants would first be planted in pots in an area of seedling benches for hardening off, following which they would be planted into the field. Stalk weight and CCS are to be measured at first harvest of the crop (the plant crop) at approximately 12 months. These measurements are to be taken in the field (at laboratories set up for juice extraction, located at each BSES station) or from material sent

to PC2 laboratories for analysis. The subsequent ratoon crop would be harvested at approximately 24 months for vegetative planting material for the following clonal assessment trial. Sugarcane is normally propagated vegetatively from lateral buds on stem pieces (setts), which establish roots and shoots once planted in a field. Once the clonal assessment trial is established the material remaining from the progeny assessment trial would be destroyed with herbicide treatment and ploughing.

91. Approximately 10% of GMOs would be selected from progeny assessment trials for the clonal assessment trials, which are intended to run in the third and fourth years of the release. Rows of clones would be propagated from stem cuttings planted directly into the field and harvested twice at approximately 12 month intervals (a plant and a ratoon crop). Measurements of cane yield and CCS would be taken at harvest. Treatments of varying nitrogen and water inputs would be carried out to assess nitrogen use efficiency and drought tolerance of lines in which these traits are expected to be enhanced. Material from selected plants would be collected for propagation for crossing, disease screening and final assessment trials. Following harvest of the ratoon crop the remaining field material would be destroyed with herbicide treatment and ploughing.

92. Approximately 1% of GMOs are to be selected for final assessment trials, which are designed to mimic commercial sugarcane cultivation. In these trials each GM line is to be grown in four-row plots for assessment of the plant and ratoon crops in the fourth and fifth years of the release. Concurrent with final assessment trials, selected GMOs are to undergo disease screening at BSES Woodford or BSES Southern. This is a requirement of the sugarcane breeding program (information supplied by the applicant). A second ratoon crop would be grown in the event that further stem material for propagation is required, however yield assessment would not be carried out.

93. The applicant proposes to cross GM sugarcane lines from six of the categories encompassing selectable marker expression (one category), altered plant growth (two categories) and altered drought tolerance (three categories, Table 4) to each other and to non-GM sugarcane cultivars. Lines for crossing are to be selected from clonal and final assessment trials, and stem material from these trials would be sent to up to two crossing facilities, at BSES Southern and BSES Meringa, for propagation. Sugarcane plants would be established from setts in non-PC2 glasshouses or on open-air seedling benches at both stations. At BSES Southern, plants would be maintained in a glasshouse until they become ready for induction of flowering, at which time they would be moved into the on-site photoperiod facility. At BSES Meringa, plants would be maintained adjacent to the on-site photoperiod facility until they become ready for induction of flowering, at which time they would be moved into the photoperiod facility. Sugarcane flowers somewhat unpredictably, and use of photoperiod facilities allows breeders to grow the plants in day lengths and temperatures most likely to induce flowering. Only those lines which flower could be used for crossing, with a maximum of 20 crosses being undertaken at each of the two BSES stations with crossing facilities in each year of the release. Inflorescences would be cut shortly before spikelets begin to open and transferred to buckets of acid in which they would be maintained until seeds develop. At BSES Meringa this would be done in a crossing shed, and at BSES Southern in the photoperiod facility. Inflorescences from the plants to be crossed would be enclosed together in bags (lanterns) which the applicant states are impermeable to pollen. Inflorescence material containing seed and flower parts (the fuzz) would be collected to muslin bags for drying. Seed may then be stored or germinated for further trials.

94. Seed from crosses would be germinated in growth cabinets (also known as germination chambers) or in glasshouses at BSES Southern and BSES Meringa and seedlings transported to other experiment stations as required for field trials. At recipient stations seedlings would be hardened in pots on seedling benches prior to planting in the field. It is proposed that

progeny from crosses would undergo a round of assessment trials similar to the original GM lines, with successive rounds of assessment trials of lines generated by crossing occurring throughout the remainder of the 15 years of the proposed release.

95. Other cultivation practices used for planting and managing the proposed trial would follow the standard practices used for commercial (non-GM) sugarcane. These are outlined in *The Biology of the* Saccharum *spp. (sugarcane)* and include compliance with Queensland Government legislation for sugarcane disease control (OGTR 2008b).

6.4 Presence of related plants in the receiving environment

96. With the exception of BSES Woodford, the other BSES stations are located in sugarcane growing districts, and commercial sugarcane crops are grown on properties adjoining the stations at a minimum distance of 20 m from the proposed field planting sites (information provided by the applicant). BSES stations are used for breeding of commercial cultivars, including field trials of non-GM material, and in addition some material from BSES stations is sent to commercial sugar mills for processing with commercial crops. The applicant states that the minimum distance between field plantings of material from the current application and other sugarcane within BSES properties would be 10 m.

97. Sugarcane is known to cross with other species within the *Saccharum* genus, however of these species only *S. spontaneum* and *S. officinarum* are reported in Australia (OGTR 2008b). Other members of the genus are maintained in various Australian germplasm collections, including a clone garden near the proposed trial site at BSES Meringa, which includes *S. officinarum, S. robustum* and *S. spontaneum* plants. Naturalised populations of *S. spontaneum* have been recorded at several locations in north Queensland, including in sugarcane growing areas along a significant part of the Mulgrave river within the Cairns LGA (Bonnett et al. 2008). Preliminary molecular analysis of this population suggested the plants have reproduced vegetatively (Bonnett et al. 2008).

98. Sugarcane has been reported to produce hybrids with a number of species within closely related genera in a group known as the *Saccharum* complex, usually under controlled experimental conditions. Genera for which hybridisation has been verified are *Erianthus* and *Miscanthus*, and these exotic species do not occur in Queensland (reviewed by Bonnett et al. 2008). Possible hybridisations to the genera *Narenga, Imperata* (blady grass), *Schlerostachya* and *Miscanthidium* have been reported, however these events have not been verified by molecular methods (reviewed by Bonnett et al. 2008).

99. Sugarcane has been reported to cross with a number of species not considered close relatives. Hybrids between commercial sugarcane and sorghum (*Sorghum bicolor*) have not been observed under natural conditions, however hybrids have been generated from experiments in which large amounts of sorghum pollen were used to pollinate *S. officinarum* flowers (reviewed by Bonnett et al. 2008). Similarly, maize (*Z. mays*) has been shown to pollinate *S. officinarum* (at very low frequency), resulting in a single confirmed hybrid plant (reviewed by Bonnett et al. 2008). According to the applicant maize and sorghum are not cultivated in close proximity to the BSES stations proposed to be used for the trial. Wild *Sorghum* species occur as widespread weeds of cultivation in Queensland. Although hybridisation between *Saccharum* and *Bambusa* (bamboo) has been reported (Rao et al. 1967), subsequent analysis has suggested the hybridisation was not genuine (Grassl 1980).

6.5 Presence of the introduced genes and RNAi constructs or similar genes and encoded proteins in the environment

100. All of the introduced genes and RNAi constructs are isolated from naturally occurring organisms, most of which are already widespread and prevalent in the environment.

101. Many of the introduced genes and gene fragments in the RNAi constructs are derived from common crop plants including bean (*P. coccineus*), barley (*H. vulgare*), rice (*O. sativa*), sugarcane and maize (*Z. mays*). Genes highly similar to the majority of these genes are also expected to occur in most other plants. Therefore, it is expected humans and other organisms routinely encounter these introduced genes and gene fragments in the RNAi constructs, and their gene products, or their homologs, through contact with plants and food.

102. Some of the introduced genes are derived from bacteria, including the common gut bacterium *E. coli*. The three marker genes *nptII*, *bla* and *uidA* are derived from *E. coli*, which is widespread in human and animal digestive systems as well as in the environment (Blattner et al. 1997), and as such it is expected to be routinely encountered by humans. Activity of the GUS enzyme encoded by *uidA* has been detected in numerous microbial, plant and animal species (Flavell et al. 1992; Gilissen et al. 1998). GUS is recognised as commonly present on fresh food. The *bla* gene confers resistance to ß-lactam antibiotics such as ampicillin. It was isolated from the bacterial Tn3 transposon. The *bla* gene would not be expressed in the GM sugarcane lines because the gene's bacterial (prokaryotic) promoter does not allow the expression in eukaryotes such as plants. The identities of some of the bacterial species from which the introduced genes were derived have been declared CCI and are not discussed further in this section.

Section 7 Australian and international approvals

7.1 Australian approvals of GM sugarcane

7.1.1 Previous releases approved by Genetic Manipulation Advisory Committee or the Regulator

103. The Regulator has previously issued licence DIR 070/2006 for the limited and controlled release of GM sugarcane containing five of the same plant growth genes (described in that application as shoot architecture genes) and the same nitrogen use efficiency gene. This licence, issued in February 2007, also includes five other genes for altered shoot architecture and three other genes for enhanced water use efficiency. The size of the release is up to 18 ha, and its duration is 3 years and 9 months.

104. In addition the Regulator has issued three other licences for the limited and controlled releases of GM sugarcane with different introduced traits. DIR 019/2002 was issued to BSES Limited for trials with GM sugarcane containing a green fluorescent reporter gene on 0.7 ha. DIR 051/2004 was issued to the University of Queensland for trials with GM sugarcane expressing sucrose isomerase over six years on an area of up to 3.55 ha per year. DIR 078/2007 was issued to the University of Queensland for trials with GM sugarcane with altered sugar production over six years on an area of up to 65 ha.

105. In addition, seven field trials were authorised under the former voluntary system that was overseen by the Genetic Manipulation Advisory Committee (GMAC): PR-23 (University of Queensland and BSES, 1993-1998) and PR-23X (University of Queensland and BSES, 1993-1994) expressing reporter genes; PR-68 (University of Queensland and BSES, 1996-2000) and PR-68X (University of Queensland and BSES, 1998-2001) modified for increased leaf scald resistance; PR-72 (BSES, 1997-2000) modified for Sugarcane mosaic virus resistance; PR-73 (CSIRO Tropical Agriculture, 1997-2000) and PR-136 (CSIRO Tropical Agriculture, 2000-2003) both modified for increased sugar yield and altered juice colour.

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

7.1.2 Approvals by other Australian government agencies

107. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. Other government regulatory requirements may also have to be met in respect of release of GMOs, including those of AQIS, FSANZ, and APVMA. This is discussed further in Chapter 3.

108. FSANZ is responsible for human food safety assessment and food labelling, including GM food. The applicant does not intend to use materials from the GM sugarcane lines in human food, accordingly an application to FSANZ has not been submitted. FSANZ approval would need to be obtained before materials from these GM sugarcane lines could be sold for use in food.

7.2 International approvals of GM sugarcane

109. There have been no releases of these GM sugarcane lines internationally. However, there have been releases of other GM sugarcane plants. The traits which have been modified include pharmaceutical protein production, virus resistance, insect pest resistance and herbicide tolerance[10].

[10]< http://www.aphis.usda.gov/brs/status/relday.html>, <http://gmoinfo.jrc.ec.europa.eu> accessed 3 April 2009.

Chapter 2 Risk assessment

Section 1 Introduction

110. Risk assessment is the overall process of identifying the sources of potential harm (hazards) and determining both the seriousness and the likelihood of any adverse outcome that may arise. The risk assessment (summarised in Figure 4) considers risks from the proposed dealings with the GMOs that could result in harm to the health and safety of people or the environment posed by, or as a result of, gene technology. It takes into account information in the application, relevant previous approvals and current scientific knowledge.

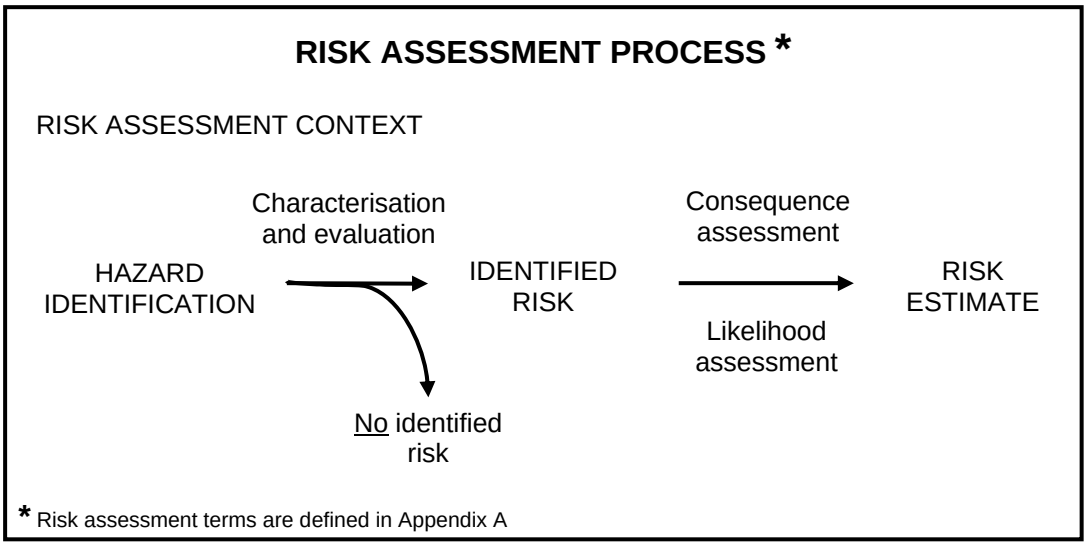

Figure 4. The risk assessment process.

111. Once the risk assessment context has been established (see Chapter 1) the next step is hazard identification to examine what harm could arise and how it could happen during a release of these GMOs into the environment.

112. It is important to note that the word 'hazard' is used in a technical rather than a colloquial sense in this document. The hazard is a source of *potential* harm. There is no implication that the hazard will *necessarily* lead to harm. A hazard can be an event, a substance or an organism (OGTR 2007a).

113. Hazard identification involves consideration of events (including causal pathways) that may lead to harm. These events are particular sets of circumstances that might occur through interactions between the GMOs and the receiving environment as a result of the proposed dealings. They include the circumstances by which people or the environment may be exposed to the GMOs, GM plant materials, GM plant by-products, the introduced genes, or products of the introduced genes.

114. A number of hazard identification techniques are used by the Regulator and staff of the OGTR, including the use of checklists, brainstorming, commonsense, reported international experience and consultation (OGTR 2007a). In conjunction with these techniques, hazards identified from previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

115. The hazard identification process results in the compilation of a list of events. Some of these events lead to more than one adverse outcome and each adverse outcome can result from more than one event.

Section 2 Hazard characterisation and the identification of risk

116. Each event compiled during hazard identification is characterised to determine which events represent a risk to the health and safety of people or the environment posed by, or as a result of, gene technology.

117. The criteria used by the Regulator to determine harm are described in Chapter 3 of the *Risk Analysis Framework* (OGTR 2007a). Harm is assessed in comparison to the parent organism and in the context of the proposed dealings and the receiving environment. Wherever possible, the risk assessment focuses on measurable criteria for determining harm.

118. The following factors are taken into account during the analysis of events that may give rise to harm:

- the proposed dealings, which may be for the purpose of experimentation, development, production, breeding, propagation, use, growth, importation, possession, supply, transport or disposal of the GMOs
- the proposed limits
- the proposed controls
- characteristics of the non-GM parent
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the biotic and abiotic environment at the site(s) of release
- agronomic management practices for the GMOs.

The nine events that were characterised are discussed in detail later in this Section. They are summarised in Table 6 where events that share a number of common features are grouped together in broader hazard categories. None were considered to lead to an identified risk that required further assessment.

119. As discussed in Chapter 1 Section 5, the GM sugarcane plants would contain combinations of the reporter gene *uidA*, and the antibiotic resistance selectable marker genes *bla* and *nptII*. The *bla* gene, encoding β-lactamase, would not be expressed in the GM sugarcane plants as it is linked to a bacterial promoter that does not function in plants. It will therefore not be further assessed for this application. The *uidA* and *nptII* genes and their products have already been considered in detail in previous RARMPs and by other regulators. Since neither of these genes has been found to pose risks to either people or the environment, their potential effects will not be further assessed for this application.

Table 6. Summary of events that may give rise to an adverse outcome through the expression of the introduced genes and RNAi constructs for altered plant growth, enhanced drought tolerance, enhanced nitrogen use efficiency, altered sucrose accumulation or improved cellulosic ethanol production from sugarcane biomass.

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.1 Production of a substance toxic/allergenic to people or toxic to other organisms	1. Exposure to GM plant material containing the introduced genes or RNAi constructs, or their end products	Allergic reactions in people or toxicity in people and other organisms	No	• The encoded proteins and their end products occur naturally in the environment and are unlikely to be toxic or allergenic to people or toxic to other organisms • None of the GM sugarcane material would be used for human food or animal feed • The limited scale, and other proposed limits and controls, further reduce exposure of people and other organisms to products of the introduced genes and RNAi constructs
Section 2.2 Spread and persistence of the GM sugarcane plants in the environment	2. Expression of the introduced genes or RNAi constructs improving the survival of the GM sugarcane plants	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• Many factors are expected to limit the spread and persistence of sugarcane in the areas proposed for release • The limits and controls proposed for the release would minimise persistence
	3. Dispersal of reproductive (sexual or asexual) GM plant materials through various means, including animals and extreme weather conditions	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• The proposed limits and controls would minimise dispersal, such as locating the sites at least 50 m from natural waterways and transporting material between BSES stations according to the Regulator's guidelines
Section 2.3 Vertical transfer of genes or genetic elements to sexually compatible plants	4. Expression of the introduced genes, RNAi constructs and regulatory sequences in other sugarcane plants	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• Pollen viability is very low under natural conditions • Pollen lanterns would reduce dispersal in glasshouses and crossing facilities • The other proposed limits and controls would also minimise gene flow • Events 1-3 did not constitute identified risks for people or the environment
	5. Expression of the introduced genes, RNAi constructs or regulatory sequences in other sexually compatible plants	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• There is limited sexual compatibility with relatives of sugarcane • Pollen lanterns would reduce dispersal in glasshouses and crossing facilities • The other proposed limits and controls would also minimise gene flow • Event 1 did not constitute identified risks for people or the environment

Hazard category	Event that may give rise to an adverse outcome	Potential adverse outcome	Identified risk?	Reason
Section 2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms	6. Presence of the introduced genes or RNAi constructs in other organisms as a result of gene transfer	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• The introduced genes and RNAi constructs and the introduced regulatory sequences are already present in the environment and are available for transfer via demonstrated natural mechanisms • Events 1 – 4 associated with expression of the introduced genes and RNAi constructs did not constitute identified risks for people or the environment
Section 2.5 Unintended changes in biochemistry, physiology or ecology	7. Changes to biochemistry, physiology or ecology of the GM sugarcane plants resulting from expression, or random insertion, of the introduced genes or RNAi constructs	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• Unintended, adverse effects, if any, would be minimised by the proposed limits and controls
Section 2.6 Unintended presence in the environment of *Agrobacterium tumefaciens* containing the introduced genes or RNAi constructs	8. Transfer of the introduced genes or RNAi constructs from *Agrobacterium* to other organisms	Weediness; allergic reactions in people or toxicity in people and other organisms	No	• Testing and antibiotic selection would be used to determine that GM plants do not contain *A. tumefaciens* cells. • It is highly unlikely that *A. tumefaciens* could conjugate with other *A. tumefaciens* strains or other bacteria naturally present in any soil that the GM plants may be grown in. • It is highly unlikely that the *A. tumefaciens* would infect other plants.
Section 2.7 Unauthorised activities	9. Use of the GMOs outside the proposed licence conditions	Potential adverse outcomes mentioned in Sections 2.1 to 2.6	No	• The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator

2.1 Production of a substance toxic/allergenic to people or toxic to other organisms

120. Toxicity is the adverse effect(s) of exposure to a dose of a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Felsot 2000).

121. Allergenicity is the potential of a protein to elicit an immunological reaction following its ingestion, dermal contact or inhalation, which may lead to tissue inflammation and organ dysfunction (Arts et al. 2006).

122. A range of organisms may be exposed directly or indirectly to the introduced genes or RNAi constructs for enhanced drought tolerance, enhanced nitrogen use efficiency, altered plant growth, altered sucrose accumulation and increased efficiency of post-harvest processing for cellulosic ethanol production and their end products. Workers cultivating the

sugarcane would be exposed to all plant parts. Organisms may be exposed directly to the introduced genes or RNAi constructs and their end products through biotic interactions with GM sugarcane plants (vertebrates, insects, symbiotic microorganisms and/or pathogenic fungi) or through contact with root exudates or dead plant material (soil biota). Indirect exposure would include organisms that feed on organisms that feed on GM sugarcane plant parts or degrade them (vertebrates, insects, fungi and/or bacteria).

Event 1. Exposure to GM plant material containing the introduced genes, RNAi constructs, or their end products

123. Expression of the introduced genes or RNAi constructs for enhanced drought tolerance, enhanced nitrogen use efficiency, altered plant growth, altered sucrose accumulation or increased efficiency of post-harvest processing for cellulosic ethanol production could potentially result in the production of novel toxic or allergenic compounds in the GM sugarcane plants, or alter the expression of endogenous sugarcane proteins. If humans or other organisms were exposed to the resulting compounds through ingestion, contact or inhalation of the GM plant materials, this may give rise to detrimental biochemical or physiological effects on the health of these humans or other organisms.

124. Non-GM sugarcane is not known to be toxic to humans or other organisms (OGTR 2008b). Although no toxicity studies have been performed on the GM sugarcane plant material, most of the introduced genes or RNAi constructs were isolated from naturally occurring organisms that are already widespread and prevalent in the environment, such as common food plants or naturally occurring bacteria (see Chapter 1, Section 6.5).

125. People and animals are exposed to most of the proteins produced by these genes through their diet and the environment. No information was found to suggest that any of the proteins encoded by the introduced genes or RNAi constructs are toxic or allergenic to people or other organisms (Chapter 1, Section 5.2.6).

126. It is not expected that any novel products would be produced as a result of the expression of the introduced gene fragments in the RNAi constructs as they are likely to be degraded upon initiating RNAi, before transcription can occur. These gene fragments are intended to silence their endogenous counterparts and therefore the level of these proteins in plant tissues would be lower than in non-GM sugarcane plants. Therefore, if these proteins had any toxicity potential then the respective sugarcane plants would be less toxic than the non-GM parent.

127. Sugarcane pollen may be an allergen (Chakraborty et al. 2001), although allergic responses to the commercial hybrid cultivars of sugarcane have not been reported in Australia. Due to the very limited quantities of pollen produced by sugarcane, it is expected that people would be exposed to very small quantities, if any, of pollen. As discussed above, the encoded proteins in the GM sugarcane are not considered to be toxic or allergenic.

128. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of exposure of people and other organisms to GM plant materials. Human contact with, or inhalation of, GM plant materials would be limited to trained and authorised staff. The proposed trial sites are located on BSES research stations so access to the general public would be minimised. There is little potential for exposure of the public to GM plant material via ingestion, skin contact or inhalation as no GM plant material would be used as animal feed or human food. Livestock would not be intentionally exposed as the GM plant material would not be used as animal feed.

129. Researchers and technical staff conducting the trials would be exposed to the GM plant materials during all phases of the trial. Workers may come into contact with the proteins encoded by the introduced genes when the plant cells have been damaged, or via pollen.

Sugarcane plants possess leaves with sharp edges and irritating hairs, and so workers typically wear appropriate clothing to reduce dermal contact. Exposure to the GM sugarcane is unlikely to lead to an adverse outcome as the GM sugarcane plants are unlikely to be any more toxic than non-GM sugarcane. No adverse effects have been reported from exposure of sugarcane workers to GM sugarcane plant material containing some of the same proteins released under licence DIR 070/2006.

130. After harvest the applicant proposes to destroy the GM sugarcane material, apart from retaining some plant material for research purposes and for new plantings within the trial. These measures would minimise exposure to the GM plant material.

131. ***Conclusion***: The potential for allergic reactions in people, or toxicity in people and other organisms as a result of exposure to GM plant materials containing proteins encoded by the introduced genes or as a result of the RNAi constructs is **not an identified risk** and will not be assessed further.

Uncertainty

132. As this is early stage research little is known about the GM sugarcane plants proposed to be released. Some of the genes and RNAi constructs have not previously been expressed in GM plants. If further information on the allergenicity or toxicity of the GM sugarcane was to become available during the proposed 15 year duration of the trial, the context of this assessment may change. Data on the toxicity or allergenicity of the GM sugarcane lines modified for altered plant growth, enhanced drought tolerance, enhanced nitrogen use efficiency, altered sucrose accumulation and increased efficiency of post-harvest processing for cellulosic ethanol production relating to toxicity and allergenicity would be able to inform future risk assessments and reduce the uncertainty associated with these genes and RNAi constructs in sugarcane.

2.2 Spread and persistence of the GM sugarcane plants in the environment

133. Baseline information on the characteristics of weeds in general, and the factors limiting the spread and persistence of non-GM sugarcane plants in particular, is given in *The Biology of the* Saccharum *spp. (sugarcane)* (OGTR 2008b). In summary, the document concludes that modern cultivars of non-GM sugarcane are not problematic weeds in Australia where sugarcane occurs almost exclusively as a managed agricultural crop.

134. Characteristics of sugarcane that may contribute to the likelihood of its persistence in the environment include its ability to regenerate by re-sprouting from underground buds or from vegetative cuttings containing viable buds. Sugarcane plants can also persist in the field for over 10 years (information supplied by the applicant).

135. Modern sugarcane cultivars are not invasive in natural undisturbed environments and are not recognised as weeds in Australia. The establishment, spread and persistence of sugarcane populations is likely to be limited by a complex interaction of factors including weed competition, pest infestation, disease infection, moisture stress and soil fertility (Bakker 1999; Hogarth & Allsopp 2000; OGTR 2008b).

136. Scenarios that could lead to increased spread and persistence of the GM sugarcane plants include expression of the introduced genes or RNAi constructs conferring tolerance to abiotic or biotic stresses, or increasing the dispersal potential of GM plant materials. These events could lead to increased exposure of vertebrates (including people), invertebrates and microorganisms to the encoded proteins.

Event 2. Expression of the introduced genes or RNAi constructs improving the survival of the GM sugarcane plants

137. If the GM sugarcane plants were to establish or persist in the environment they could increase the exposure of humans and other organisms to the GM plant material. The potential for increased allergenicity in people or toxicity in people and other organisms as a result of contact with GM plant materials has been considered in Event 1 and was not considered an identified risk.

138. If the expression of the introduced genes or RNAi constructs for enhanced drought tolerance, enhanced nitrogen use efficiency, altered plant growth, altered sucrose accumulation or increased efficiency of post-harvest processing for cellulosic ethanol production were to provide the GM sugarcane plants with a significant selective advantage over non-GM sugarcane plants and they were able to establish and persist in favourable non-agricultural environments, this may give rise to lower abundance of desirable species, reduced species richness, or undesirable changes in species composition. Similarly, the GM sugarcane plants could adversely affect agricultural environments if they exhibited a greater ability to establish and persist than non-GM sugarcane.

139. The impact of the genetic modifications on survival of the GM sugarcane plants is uncharacterised under field conditions. However, a number of predictions can be made based on knowledge of the gene functions and prediction of their effects when expressed in the GM plants. Predictions can also be made based on the observed phenotypes of some of the GM sugarcane lines released under licence DIR 070/2006, which contain similar constructs to some in the current application. The phenotypes of these lines are summarised in Chapter 1, Section 5.5.2.

140. Two of the genes in the current application are GA20-oxidases, which are enzymes catalysing a step in GA biosynthesis leading to production of biologically active GA. Increasing GA20-oxidase expression in potato, *Arabidopsis*, aspen and tobacco results in elongation of internodes, early flowering, taller plants and increased biomass (Coles et al. 1999; Carrera et al. 2000; Eriksson et al. 2000; Biemelt et al. 2004). Silencing of GA20-oxidases has generally opposite effects in *Arabidopsis* and potato, including decreased internode elongation, reduced stem elongation and late flowering (Coles et al. 1999; Carrera et al. 2000). Over-expression of a pumpkin GA20-oxidase in *Arabidopsis*, lettuce and *Solanum dulcamara* has been shown to have effects similar to those seen for GA20-oxidase silencing (Curtis et al. 2000; Niki et al. 2001; Radi et al. 2006). A third GA biosynthesis gene in the current application is a GA2-oxidase, which decreases levels of biologically active gibberellins. Over-expression of GA2-oxidase in *Arabidopsis*, tobacco, *Nicotiana sylvestris* and rice has been shown to result in dwarfism, reduced germination and delayed flowering (Sakamoto et al. 2003; Biemelt et al. 2004; Lee & Zeevaart 2005; Radi et al. 2006). These phenotypes are highly similar to the effects of reduced GA20-oxidase expression, highlighting the opposite biological functions of these two enzymes. Under DIR 070/2006, lines were released containing the same GA oxidases as are included in the current application.

141. The other genes to be used to alter plant growth in the GM sugarcane plants are homologs of *Teosinte Branched1*. Over-expression of the endogenous *OsTB1* gene in rice led to a reduction in tillering while the converse phenotype resulted from silencing of the endogenous gene (Takeda et al. 2003). Similarly, over-expression of maize *TB1* in GM wheat plants produced shorter plants with a reduced number of tillers and spikes but an increased number of leaves (Lewis et al. 2008). Sugarcane lines generated under DIR 070/2006 with increased *OsTB1* or *ShTB1* (named *SoTB1* in that application) showed no alteration in phenotype, whereas lines containing RNAi constructs reducing *ShTB1* expression were shorter with thinner stalks and increased tiller production (information provided by applicant).

142. Alteration of plant growth may result in improved competitiveness of the GM sugarcane compared to non-GM sugarcane. Potential changes could include: more vigorous growth improving the ability of sugarcane to establish in a competitive environment; increased plant height improving the ability of sugarcane to shade out other plants; shorter stature resulting in a decreased tendency to lodge; altered bud growth characteristics leading to stem pieces more readily shooting and establishing as new plants. Secondary effects of these changes, including potential effects on reproductive behaviour, are considered below. These changes could increase the potential weediness of the GM sugarcane plants.

143. One of the categories of GM sugarcane plants contains the *ZmDof1* gene for enhanced nitrogen use efficiency, which if successful would confer improved growth in soil with low nitrogen levels. Expression of *ZmDof1* in *A. thaliana* and potato has been shown to improve growth under low nitrogen conditions in the laboratory (Yanagisawa et al. 2004; Yanagisawa 2004). In an environment in which nitrogen availability was the main factor limiting the spread and persistence of sugarcane, expression of this gene for nitrogen use efficiency could increase weediness of the GM sugarcane plants. Dof proteins have also been shown be involved in numerous other processes including light responses, auxin responses, defence and seed germination (reviewed by Yanagisawa 2002). Indeed, an *Arabidopsis* mutant with a disrupted *Dof* gene (*DAG1*) produced seed with no dormancy and no requirement for light to induce germination (Papi et al. 2000). These mutants also had altered seed coat structure (Papi et al. 2002).

144. In environments where nitrogen availability is limiting, improved nitrogen use efficiency could increase the competitiveness and increase the potential weediness of the GM sugarcane plants compared to non-GM sugarcane.

145. Seven of the categories of GM sugarcane plants contain introduced gene constructs derived from five genes designed to alter sucrose accumulation. The identities of these genes has been declared CCI and they are not discussed further in this section

146. It could be speculated that changes in sucrose accumulation could improve the competitiveness of the GM sugarcane compared to non-GM sugarcane, increasing its potential weediness. For example, plant growth requires sucrose as an energy source, and so altered sucrose accumulation could alter plant growth, potentially leading to some of the effects considered above for GM lines in which plant growth may be altered. However, the introduced gene fragments are currently poorly characterised, and it is highly speculative to consider their potential secondary effects.

147. Six of the categories of GM sugarcane plants contain introduced gene constructs designed to increase the efficiency of post-harvest processing of sugarcane biomass for cellulosic ethanol production. The identities of these genes has been declared CCI and they are not discussed further in this section

148. Three of the categories of GM sugarcane plants contain introduced gene constructs for enhanced drought tolerance. In an environment in which water availability was the main factor limiting the spread and persistence of sugarcane, expression of the genes for enhanced drought tolerance could result in increased weediness of the GM sugarcane plants. The identities of two of these genes, *WUE1* and *WUE2* has been declared CCI and they are not discussed further in this section.

149. Expression of *OsDREB1A* and closely related genes from rice and *Arabidopsis* has been studied in a number of GM plants where improvements to drought tolerance have been shown. *OsDREB1A* and other rice DREB genes have been shown to be induced by dehydration, cold stress and high salt (Dubouzet et al. 2003), and are thought to play a role in initiating transcriptional responses to stress. Over-expression of *OsDREB1A* in *Arabidopsis*

and rice plants leads to improved drought, salt and freezing tolerance, and growth retardation (Dubouzet et al. 2003; Ito et al. 2006). GM *Arabidopsis* plants expressing the *Arabidopsis DREB1A* gene from a constitutive promoter showed improved tolerance of drought, salinity and freezing stress, and severe growth retardation (Liu et al. 1998; Kasuga et al. 1999; Ito et al. 2006). However, when *AtDREB1A* was expressed from a stress-inducible promoter plant growth was normal and tolerance of stress was further improved (Kasuga et al. 1999).

150. Through unintended effects, the GM sugarcane plants may have increased weediness. For example, plant responses to a variety of stresses involve interconnected signalling and transcriptional controls. Homologues of the introduced genes for enhanced drought tolerance encode proteins which have been shown to enhance tolerance to other abiotic and biotic stresses such as cold, freezing, pathogens and salinity. Thus, it is possible that the introduced genes and gene fragments may give rise to GM sugarcane plants with enhanced tolerance of a range of stresses. The introduced genes or RNAi constructs, particularly those attempting to alter plant growth, could potentially affect fertility, flowering time and seed development (including germination) of the GM sugarcane lines as compared to commercially grown sugarcane, which could lead to increased spread and persistence of the GM sugarcane plants.

151. During the trial the applicant proposes to cross some of the GM plants to produce offspring containing more than one of the categories of genetic modification. This may create a plant with more than one gene for enhanced drought tolerance, or altered plant growth, or genes for both of these traits. This may result in a plant that has enhanced stress tolerance and earlier or increased flowering or seed production.

152. However, two issues should be considered. First it should be noted that the anticipated effects of the some of the introduced genes or RNAi constructs are derived from the available published literature on the genes and related members of their gene families. Thus, a much broader range of anticipated effects are considered than would likely result from any single introduced gene. A second consideration is that when a gene is expressed in different plant species the same effect on phenotype does not always eventuate. Therefore, the introduced genes or RNAi constructs may not confer any phenotypic changes or enhanced stress tolerance in the GM sugarcane plants.

153. It is possible that altered expression of a regulatory gene involved in plant responses to stress could enhance tolerance to several environmental stresses. However, it is unlikely that the introduced genes or RNAi constructs for enhanced drought tolerance, enhanced nitrogen use efficiency, altered plant growth, altered sucrose accumulation or increased efficiency of post-harvest processing for cellulosic ethanol production could alter all of the characteristics which limit the spread and persistence of sugarcane such as its low fertility and seed viability, poor ability of seedlings to establish and compete without human intervention, nutrient requirements, and susceptibility to pests and diseases and other factors that normally limit the spread and persistence of sugarcane plants in Australia (Bakker 1999; Hogarth & Allsopp 2000; OGTR 2008b).

154. Although alteration of plant growth by the introduced genes or RNAi constructs could potentially result in increased weediness of the GM sugarcane plants, it could also result in GM plants of lower fitness than other commercially available sugarcane varieties because of unintended effects of the introduced genes or RNAi constructs. For example, lines released under DIR 070/2006 expressing *HvGA20ox-1* and-*2* displayed increased plant height which in turn results in an increased susceptibility to lodging. In the same release, plants expressing *PcGA2-ox1* had decreased plant height, and the applicant states that they may be less competitive as a result, due to being shaded more readily by competition. The trial would enable the applicant to assess the effect of the introduced genes or RNAi constructs on plant physiology and agronomic performance.

155. A further important consideration is the reduction in plant vigour routinely observed in sugarcane plants which have undergone tissue culture. The current application includes two categories of GM sugarcane expressing only marker genes, for the specific purpose of providing a baseline against which effects of the other genetic modifications may be measured, as comparison to non-tissue-cultured sugarcane is inappropriate. Data provided by the applicant from release DIR 070/2006 show that plant height, stalk number, stem diameter and cane yield are reduced in a population of *nptII*-expressing GM sugarcane compared to untransformed sugarcane. These effects are expected to generally decrease the competitiveness of GM sugarcane, in some cases by a substantial margin.

156. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would minimise the likelihood of the spread and persistence of the GM sugarcane plants proposed for release. The release would be of limited size and at a limited number of locations. However, the proposed duration of the release gives rise to some uncertainty in the risk assessment (see below and Chapter 3, Section 4.1.1). The applicant proposes a number of control measures, including destruction of all plant materials not required for further analysis, and post harvest monitoring of the proposed sites for at least one year and until no sugarcane plants have been found on the sites for six months.

Conclusion: The potential for increased weediness, allergenicity or toxicity due to expression of the introduced genes or RNAi constructs for enhanced drought tolerance, enhanced nitrogen use efficiency, altered plant growth, altered sucrose accumulation or increased efficiency of post-harvest processing for cellulosic ethanol production improving the survival of the GM sugarcane plants is **not an identified risk** and will not be assessed further.

Uncertainty

157. This field trial is part of early stage research, and therefore the effect of the introduced genes or RNAi constructs for altered plant growth, altered sucrose accumulation or increased efficiency of post-harvest processing for cellulosic ethanol production on the GM sugarcane lines is unknown in the field. The properties of progeny of crosses between the GM sugarcane and different categories of GM sugarcane or non-GM sugarcane different to the parental cultivar, are also unknown. Also, the ability of the GM sugarcane plants to withstand drought stress or have enhanced nitrogen use efficiency throughout different stages of their lifecycle, as compared to commercially available sugarcane cultivars, is unknown. Under current agricultural practices, climate and weather patterns the proposed controls are appropriate, however, over time if these were to change then the appropriateness of these controls is less certain. Data on the unintentional effects of the genes for altered plant growth, altered sucrose accumulation, increased efficiency of post-harvest processing for cellulosic ethanol production, enhanced drought tolerance and enhanced nitrogen use efficiency, particularly that relating to weediness, would inform future risk assessments and reduce the uncertainty of assessing risks associated with dealings with GM sugarcane containing these genes and RNAi constructs.

Event 3. Dispersal of reproductive (sexual or asexual) GM plant materials through various means, including animals and extreme weather conditions

158. If the GM sugarcane plants were to be dispersed from the release sites they could increase the exposure of humans and other organisms to the GM plant material and/or establish and persist in the environment. The effects of contact, inhalation or ingestion of the GM sugarcane plants have been assessed in Event 1 and were not an identified risk. The potential for the introduced genes or RNAi constructs to result in improved survival of the GM sugarcane plants in the environment was assessed in Event 2 and was not an identified risk.

159. Although sugarcane can produce seeds, commercial sugarcane is propagated vegetatively, with stem pieces (setts) being planted in the field. Usually one bud per stem cutting is developed into a primary stalk which then gives rise to tillers (for more details see *The Biology of the* Saccharum *spp. (sugarcane)* (OGTR 2008b).

160. Viable sugarcane stems could be unintentionally dispersed during transportation. Sugarcane volunteers have been found growing along roadsides and railways in sugarcane cultivation areas. These volunteers are believed to have originated from stem cuttings displaced or fallen from vehicles, and generally consist of only a few groups of stems which do not become self perpetuating or result in further spread.

161. In the course of the dealings the applicant proposes to transport GM sugarcane setts between the release sites, cultivate GM sugarcane plants and collect GM plant materials for research purposes, laboratory research or new plantings within the trial. Accidental spillage or dispersal of GM plant materials, especially setts, in the course of these dealings could allow the GM sugarcane plants to spread and persist in the environment.

162. The applicant has proposed to transport the GM sugarcane plants and setts to and between BSES stations according to transport guidelines issued by the Regulator. The applicant has also proposed that transport of harvested material between field locations and crushing machinery within BSES stations would be in covered trailers, with sugarcane plant material being tied down. This constitutes a lower level of containment than is required by the Regulator's transport guidelines, which require propagative material be contained within primary and secondary unbreakable containers. A standard condition of DIR licences requiring that all transport be according to the Regulator's transport guidelines is included in the imposed licence conditions.

163. Spillage of setts during transport according to the Regulator's transport guidelines would be rare, reducing the risk of dispersal of the GM sugarcane during transport. Further, the accounting procedures required by the Regulator's guidelines would have the result that any spillage would be detected, initiating cleaning and monitoring of the site of the spill. Finally, appropriate environmental conditions are necessary for survival and persistence of any distributed setts. For example, soil-borne fungal infections are known to reduce sett germination, leading to the common practice of treating setts with fungicide prior to planting (FAO 2004).

164. During harvesting of GM sugarcane, accidental mixing of non-GM sugarcane with GM sugarcane may occur. At the field sites, the applicant proposes to separate GM sugarcane from any adjacent sugarcane by a guard row of non-GM cane and a 6 m isolation zone of bare ground or grass. This measure would clearly separate the GM sugarcane from neighbouring non-GM sugarcane, minimising the likelihood of accidental mixing at harvest. Additionally, the applicant has proposed to mark GM field corners with star pickets, sign GM plantings, and implement staff management procedures. In crossing facilities, the applicant proposes to label GM sugarcane with barcodes, and maintain GM sugarcane in clearly signed areas separate to areas used for non-GM sugarcane. These measures would minimise any potential mixing of non-GM sugarcane with GM sugarcane.

165. The applicant has proposed to cultivate GM sugarcane plants on seedling benches prior to planting in the field, using areas set aside at each BSES station at which field planting would occur. In these nursery facilities non-GM sugarcane plants would also be cultivated on separate benches to the GM sugarcane, and the GM sugarcane would be identified by barcode labels. Separation of GM and non-GM sugarcane within the nursery area and identification of GM plants would reduce the potential for human error leading to accidental dispersal of the GM sugarcane by workers mistaking them for non-GM plants.

166. The applicant proposes to thoroughly clean equipment used to plant and the harvest the GM sugarcane to prevent dispersal of GM plant materials to other locations and to meet domestic quarantine requirements. The applicant proposes to destroy all plant material other than that collected for future research or for new plantings within the proposed release. The sites would be monitored for volunteers after the final harvest and any volunteer sugarcane plants would be destroyed.

167. On completion of the trial the sugarcane plant material would be destroyed by a combination of harvesting, mulching, herbicide treatment and burning. The applicant has proposed that some material be left to rot in the field. This could lead to the possibility of dispersal of potentially viable stem pieces which may lead to the establishment of GM sugarcane.

168. Flooding may cause dispersal of plant parts. However, control measures have been proposed by the applicant to minimise dispersal by flooding. These include locating the proposed release sites at least 50 m away from natural waterways (with the exception of the BSES Meringa photoperiod facility) on land that is at minimal risk of flooding. The applicant states that BSES Meringa, Burdekin and Southern have no history of flooding, and BSES Woodford is in an elevated part of a flood-prone area and has experienced no significant flooding in the past 10 years. At BSES Woodford the applicant plans to use the highest part of the site for the trial, which has no history of flooding. The applicant states that flooding is rare at BSES Central, which is 5 km from the nearest major waterway and that the station was unaffected by significant floods in the area in the high rain experience in the most recent rainy season. The applicant also states that sugarcane plants are generally not uprooted by floodwaters, unless significant water flow occurs. Although the BSES Meringa photoperiod facility is within approximately 20 m of a small natural waterway, the risk of dispersal of sugarcane from this facility is limited because GM sugarcane at this location would be large plants in pots held within a large trolley (which would restrict movement of pots), and in addition BSES Meringa has no history of flooding. All other parts of the proposed crossing facility at BSES Meringa are more than 50 m from natural waterways.

169. As sugarcane can reproduce through vegetative cuttings, it is possible that pests such as feral pigs or other large animals could disperse viable materials. However, this is unlikely to occur because of the size and weight of the canes. Dispersal of cane material through these means has not been reported to date.

170. The sexual reproductive behaviour of sugarcane has been little studied in the field, as seed production is not important for sugarcane cropping. Conditions for seed production have been studied in breeding facilities, however, the relevance of these observations to field-grown sugarcane is limited by the optimisation of environmental conditions in breeding situations. Sugarcane plants can flower and produce seed in the field where appropriate conditions exist for floral induction and production of viable pollen. These factors are discussed further in Event 4. To summarise, the frequency of production of fertile seed decreases with increasing distance from the equator. BSES Meringa is the proposed location at which production of viable seed is most likely, and this station is not proposed to be used for field trials. The applicant proposes to monitor GM sugarcane at BSES Meringa for flowering, and contain inflorescences in pollen lanterns prior to spikelets opening to control distribution of pollen.

171. Sugarcane seed is adapted for wind dispersal by the retention of callus hairs (or fuzz) (Babu 1979). Sugarcane seed is short-lived, losing 90% of viability within 80 days at 28ºC unless it is desiccated (Rao 1980), with more recent data suggesting that seed remains viable for at least 2-3 months when stored at room temperature (Powell et al. 2008). Numerous animals, including mammals, birds and insects, are known to eat GM sugarcane plant

materials, including flowers, pollen and seed. Of those, seed has the potential to produce a new GM sugarcane plant if it were still viable after excretion.

172. Generally, sugarcane seeds are hard to germinate and the seedlings require particular favourable environmental conditions to survive for the first three to four weeks after germination (Breaux & Miller 1987) and therefore require careful nurturing. Powell et al. (2008) studied the effect of temperature on sugarcane seed germination, finding that germination occurred at temperatures from 15 to 42^{o}C, with optimum germination at 30 to 36^{o}C. Combining this data with knowledge of seed longevity, the authors concluded that sugarcane seed, which is predominantly produced in winter, could survive until temperatures favoured germination only in north Queensland. Seedlings are occasionally observed in the field in the Herbert district and further north (Robert Birch, personal communication, 2008). A requirement for very specific environmental conditions for germination is suggested by the lack of reports of seed germination in more southern field sites, and by reported low numbers of seedlings observed in more northern regions. It is unknown whether field-germinated seedlings survive to maturity. Of the BSES stations proposed to be used for the trial, only BSES Meringa is located in an area where temperatures are thought to be conducive to sugarcane seed germination in the field. Within the proposed crossing facility at BSES Meringa the applicant proposes to contain open inflorescences in pollen lanterns and mature fuzz containing seeds in bags for drying seed, and so the dispersal of pollen or seed into the environment is considered very unlikely.

173. There is no data to suggest that seed viability or dispersal would be altered in the GM sugarcane plants compared to the non-GM parental sugarcane plants. However, seed production may be increased in some of the GM sugarcane plants compared to the non-GM parental sugarcane plants (see Event 2). Survival of any GM sugarcane seedlings would be limited by factors such as humidity, temperature, low intrinsic competitive ability, nutrient availability, pests and diseases and other environmental factors that normally limit the spread and persistence of sugarcane plants in Australia (see Event 2).

174. In the unlikely event that material is dispersed away from the proposed release sites, it is unlikely to be a source of potential harm because the GM plants are unlikely to establish and persist outside the release sites or to be toxic or allergenic (Events 1 and 2).

175. ***Conclusion:*** The potential for allergenicity, toxicity or increased weediness due to the dispersal of reproductive (sexual or asexual) GM plant materials through various means including animals and extreme weather conditions is **not an identified risk** and will not be assessed further.

Uncertainty

176. The assessment of dispersal of reproductive plant material has been prepared using the current context for the trial described in Chapter 1, including weather conditions and agricultural practices. This may alter over the proposed fifteen year period of the release. For example, cultivars used in the Queensland sugarcane industry have recently been changing in response to occurrence of smut. While there is published literature relating to older cultivars, there is less industry experience and published knowledge of flowering, seed production and seed viability of new cultivars. In addition, more cultivars are anticipated to be introduced in the near future. In this potentially changing context, it is uncertain whether parental non-GM sugarcane cultivars which may be used in the current application have an altered ability to disperse and whether the addition of any of the proposed genetic modifications will give an additional selective advantage.

2.3 Vertical transfer of genes or genetic elements to sexually compatible plants

177. Vertical gene flow is the transfer of genetic information from an individual organism to its progeny by conventional heredity mechanisms, both asexual and sexual. In flowering plants, pollen dispersal is the main mode of gene flow (Waines & Hedge 2003). For GM crops, vertical gene flow could therefore occur via successful cross-pollination between the crop and neighbouring crops, related weeds or native plants (Glover 2002).

178. Baseline information on vertical gene transfer associated with non-GM sugarcane plants can be found in *The Biology of the* Saccharum *spp. (sugarcane)* (OGTR 2008b). In summary, sugarcane pollen viability is extremely low under natural conditions, commercial sugarcane varieties show very low fertility and crossing to plants outside of the *Saccharum* genus has rarely been observed. Thus, it is highly unlikely that crossing with sexually compatible plants would occur.

Event 4. Expression of the introduced genes or RNAi constructs and regulatory sequences in other sugarcane plants

179. Transfer and expression of the introduced genes or RNAi constructs for enhanced drought tolerance, enhanced nitrogen use efficiency, altered plant growth, altered sucrose accumulation or increased efficiency of post-harvest processing for cellulosic ethanol production to other sugarcane plants could increase the weediness potential, or alter the potential allergenicity and/or toxicity of the resulting plants.

180. All of the introduced regulatory sequences are expected to operate in the same manner as regulatory elements endogenous to the sugarcane plants. While the transfer of either endogenous or introduced regulatory sequences could result in unpredictable effects, the impacts from the introduced regulatory elements are likely to be equivalent and no greater than the endogenous regulatory elements.

181. As discussed in Event 1, allergenicity to people and toxicity to people and other organisms are not expected to be changed in the GM sugarcane plants by the introduced genes or RNAi constructs. This will be the same if the introduced genes or RNAi constructs are expressed in other sugarcane plants.

182. Sugarcane is principally a wind pollinated out-crosser with a low frequency of self pollination. The initiation of flowering is strongly influenced by day length and other environmental conditions. Pollen viability and pollen movement are also strongly influenced by environmental conditions including temperature, relative humidity and wind intensity (discussed below). There is little information available on rates and distances of outcrossing for sugarcane.

183. Sugarcane is cultivated within close proximity to all of the proposed trial sites. At BSES Woodford, Southern, Central and Burdekin, non-GM sugarcane is cultivated within the BSES stations at a minimum distance of 10 m from the proposed trial sites. At BSES Southern, Central and Burdekin, commercial sugarcane is propagated in adjacent properties at a minimum distance of 20 m from the proposed trial sites. Within crossing facilities at BSES Southern and Meringa, the applicant proposes to enclose open GM sugarcane inflorescences in pollen-impermeable lanterns to separate them from non-GM sugarcane inflorescences. The genes for enhanced drought tolerance, enhanced nitrogen use efficiency, altered plant growth, altered sucrose accumulation or increased efficiency of post-harvest processing for cellulosic ethanol production could be transferred to these sexually compatible sugarcane plants.

184. Sugarcane does not flower with great consistency, and cultivars differ in their propensity to flower (Bonnett et al. 2007). The initiation of sugarcane flowering has largely been studied within the context of breeding programs, where unreliable flowering of many cultivars is a

reported problem (Berding et al. 2004). Observations of the breeding collection at BSES Meringa indicated that an average of 38.2% of clones in the breeding collection flowered each year between 1978 to 1996 and the flowering was variable, ranging from 16% of clones in 1993 to 66% in 1984 (Cox et al. 2000). Shortening day length appears to control the initiation of flowering (Moore & Nuss 1987) and environmental factors appear to influence the extent of flowering. Under adequate irrigation, temperatures above 32^{o}C impede flowering (Cox et al. 2000), and night-time temperatures above 18^{o}C are required for floral development (Berding 1981). Sugarcane has been observed to flower throughout Queensland and as far south as the New South Wales border (Graham Bonnett and Juan Jose Olivares-Villegas, personal communication 2009), however the frequency of flowering in the Burdekin region and further south, which is generally thought to be low, has not been systematically recorded.

185. Sugarcane pollen viability is variable and is strongly influenced by temperature, being greatly reduced under night-time temperatures below 21^{o}C (Berding 1981). In Queensland, this leads to a general reduction in levels of pollen viability the further south sugarcane is cultivated. The applicant has provided data showing that pollen viability on inflorescences sampled from commercial crops in the Mulgrave region was significantly higher than in samples from the Burdekin region, which showed very low pollen viability. Bonnett et al. (2007) also reported low levels of pollen viability in the Burdekin region. Viable pollen has been sampled from field-grown sugarcane from Bundaberg and at the New South Wales border (Graham Bonnett and Juan Jose Olivares-Villegas, personal communication 2009). Reports of seed germination from fuzz collected from sugarcane fields show that very low amounts of viable seed are produced in the Burdekin region (Bonnett et al. 2007), in Mackay (data provided by the applicant), and as far south as Bundaberg (Graham Bonnett and Juan Jose Olivares-Villegas, personal communication 2009). However, there are no reports of systematic field observations of the production of viable pollen or seed in the majority of sugarcane-growing regions, and how this is effected by seasonal variability. There is a particular lack of relevant data for areas south of the Burdekin region. Available reports reveal variability between sugarcane cultivars, and strongly indicate that the frequency of flowering and production of viable pollen is very low in most parts of Queensland, with sugarcane seed production most likely to occur north of the Burdekin region. Sugarcane pollen rapidly desiccates and is not viable beyond 20 minutes in open air (Venkatraman 1922) or 35 minutes at 26.5ºC and 65% relative humidity (Moore 1976). Standard cultivation practices for commercial sugarcane cultivation include harvesting prior to flowering to maximise sugar content. Although this practice is not strictly adhered to, it would limit the opportunity for gene flow to nearby commercial crops.

186. As discussed in Event 3, sugarcane seed remains viable for only several months (Rao 1980; Powell et al. 2008), and germination is unreliable (Breaux & Miller 1987). Survival of any GM sugarcane seedlings would be limited by factors that normally limit the spread and persistence of sugarcane plants in Australia.

187. North of the Burdekin region (ie at BSES Meringa) environmental conditions can be suitable for viable pollen production, seed germination and seedling survival. In these regions the GM sugarcane plants would be grown in pots and monitored for flowering. The applicant proposes that flowering plants would have their inflorescences removed shortly before spikelet opening, at which time the inflorescences would be transferred to a crossing shed, where they would be enclosed in pollen lanterns. The applicant has also proposed measures to control potential pollen transfer in the event that spikelets open prior to inflorescences being enclosed in pollen lanterns, including removal and destruction of any spikelets which open before pollen lanterns are applied to GM inflorescences, and removal and destruction of any open spikelets on nearby non-GM inflorescences which are not enclosed in pollen lanterns, preventing set of seed. These measures would minimise the dispersal of pollen and the

potential for cross-pollination to lead to seed set, thus limiting potential gene flow to other sugarcane plants in crossing facilities.

188. During the trial the applicant proposes to cross some of the GM sugarcane plants to produce offspring containing more than one of the sugarcane genetic modifications, using plants from categories expected to have altered drought tolerance and altered plant growth. At BSES Woodford, Southern and Meringa, GM sugarcane may be grown under the limited and controlled releases DIR 070/2006 and DIR 078/2007, and at BSES Central under DIR 078/2007. These GM sugarcane plants contain introduced genes or RNAi constructs for altered plant growth, enhanced nitrogen use efficiency and altered sugar production. Nine of the eighteen genes in DIR 070/2006 are the same as used in the current application, in which their expression is controlled by a greater variety of promoters and terminators.

189. Low levels of gene flow both within and between the sugarcane trials, or deliberate experimental crossing within the proposed trial, may lead to stacking of GM traits, thus producing a GM sugarcane plant which possesses genes for enhanced drought tolerance, enhanced nitrogen use efficiency, altered plant growth, altered sucrose production, altered sucrose accumulation and increased efficiency of post-harvest processing for cellulosic ethanol production which may give rise to altered weediness compared to any individual GM sugarcane plant in the proposed release.

190. However, the combination of traits is likely to contribute only incrementally to the potential weediness of the GM sugarcane plants, the spread and persistence of which would be limited by factors such as low fertility and seed viability, poor ability to establish and thrive without human intervention, competition with other plants, soil type and fertility, and pests and diseases that normally limit the spread and persistence of sugarcane plants in Australia.

191. The proposed limits and controls of the trial (Chapter 1, Sections 3.2 and 3.3) would restrict the potential for pollen flow and gene transfer to non-GM sugarcane plants. The applicant also proposes to perform post harvest monitoring of the field planting sites for at least twelve months and until the sites have been clear of volunteers for six months and to destroy any volunteer plants found at the sites. This would ensure that any GM sugarcane seeds or plants that were potentially the product of gene flow remaining in these areas would be destroyed.

192. ***Conclusion:*** The potential for allergenicity in people, or toxicity in people and other organisms or increased weediness due to the expression of the introduced genes, RNAi constructs and regulatory sequences in other sugarcane plants as a result of gene transfer is **not an identified risk** and will not be assessed further.

Uncertainty

193. As this is early stage research no characterisation of the GM sugarcane plants has been performed. The secondary effects of genes, and combinations of genes, is not known and it is possible that they may affect flowering, pollen production or seed viability. This could lead to a greater chance of gene flow in the future. New varieties of sugarcane may also be introduced over the long term of this application which may have altered flowering characteristics, especially in cooler climatic conditions. Predictions of how the climate may change over the proposed 15 year duration of this release suggest that changes in the reproductive capacity of sugarcane in the locations of the proposed release may occur over this period.

Event 5. Expression of the introduced genes, RNAi constructs and regulatory sequences in other sexually compatible plants

194. Sugarcane is a hybrid derived from *S. spontaneum* and *S. officinarum*, and these species as well as other members of the *Saccharum* genus are sexually compatible with commercial sugarcane hybrids. Sugarcane is also sexually compatible with other genera within the tribe Andropogoneae (for more detail see Chapter 1, Section 6.4) (OGTR 2008b). To summarise, genera for which hybridisation to sugarcane has been observed under experimental conditions are *Erianthus, Miscanthus*, *Sorghum* and *Zea* (maize). Possible artificial hybridisations to the genera *Narenga, Imperata* (blady grass)*, Sclerostachya* and *Miscanthidium* have been reported, but not fully verified. Although hybridisation with *Bambusa* (bamboo) has been reported, this report is thought to be false.

195. Commercial sorghum and maize crops are not currently cultivated near the BSES stations at which trial sites are proposed (information supplied by applicant), however, for the planned station (for which the location is yet to be determined) proximity to sorghum and maize cultivation is unknown, as is the proximity of existing BSES stations to future maize and sorghum plantings. Wild sorghum species are weeds of Australian sugarcane crops (McMahon et al. 2000) and are widespread in Australia (Hnatiuk 1990).

196. Blady grass (*Imperata cylindrica*) is common throughout Queensland coastal areas, however the applicant has stated that no-one is aware of the presence of much (if any) blady grass in the vicinity of the BSES stations proposed to be used for the trial. The presence of blady grass at the planned BSES station proposed to be used in the release is unknown, as is the future distribution of this grass.

197. Although some other sexually compatible species are likely to occur in the areas of the proposed release, crosses of sugarcane with genera outside the *Saccharum* genus are extremely difficult even under experimental conditions. The extremely low numbers of known genuine progeny obtained in any crosses have been of low vigour and sterile. A hybrid between maize and sugarcane has been generated using maize pollen (Janakiammal 1938 as cited by Bonnett et al. 2008) and confirmed using molecular markers (Nair et al. 2006). However, this cross has proved difficult to replicate (Janaki-Ammal 1941). Hybrids between sorghum and sugarcane have been generated using sugarcane as both the female (Grassl 1980) and male parent (Nair 1999). However, these experiments used large numbers of florets and produced few hybrids which were male sterile (Grassl 1980) or lacked vigour and showed slow growth (Nair 1999). Hybrids with *Imperata cylindrica* have been reported from a controlled cross, but have not been confirmed by molecular analysis (Bonnett et al. 2008).

198. In one of the areas proposed for release (BSES Meringa), *S. spontaneum*, *S. robustum* and *S. officinarum* are grown as part of a germplasm collection (see Chapter 1, Section 6.4). These species are all sexually compatible with cultivated sugarcane. *S. robustum* is thought to be the ancestral species from which *S officinarum* is derived (D'Hont et al. 1998; Brown et al. 2007), and is so closely related that it has been proposed that it should be classified as *S. officinarum* (Irvine 1999). The potential for GM sugarcane in the proposed release to cross-pollinate these plants would be minimised by measures proposed by the applicant to control pollen dispersal, in particular, enclosure of inflorescences of GM sugarcane in pollen lanterns prior to pollen shed.

199. Naturalised populations of *S. spontaneum* have been recorded at several locations within sugarcane growing areas and along part of the Mulgrave river within the Cairns LGA (Bonnett et al. 2008). Naturalised *S. spontaneum* is considered the most likely species to naturally hybridise with cultivated sugarcane (Bonnett et al. 2008), and this appears to be the case for the GM sugarcane lines in the current application. However, recorded naturalised populations of *S. spontaneum* on the Mulgrave River are many kilometres from the nearest of

the BSES stations proposed for the release, BSES Meringa (information supplied by applicant).

200. Hybridisation would require synchronicity of flowering between the GM sugarcane plants and related species to enable cross-pollination and gene flow to occur. *S. spontaneum* has been shown to flower synchronously with sugarcane in the Herbert River region (near Ingham), and predominantly asynchronously in the Mulgrave Region (near BSES Meringa, Olivares-Villegas et al. 2008). *S. spontaneum* is not known to occur in proximity to the more southern sites of the proposed release (at BSES Woodford, Southern, Central and Burdekin).

201. Expression of the introduced genes or RNAi constructs in other sexually compatible plants is also unlikely to give these plants a significant selective advantage. The proposed limits and controls of the trial (Chapter 1, Sections 0 and 3.3) would restrict the potential for pollen flow and gene transfer to sexually compatible plants. At the proposed release site in the Mulgrave region (BSES Meringa) the applicant proposes to move the inflorescence of flowering plants into a crossing shed prior to flowering and enclose the inflorescences with pollen-proof lanterns to reduce pollen dissemination.

202. ***Conclusion:*** The potential for allergenicity in people, or toxicity in people and other organisms or increased weediness due to the expression of the introduced genes, RNAi constructs and regulatory sequences in other sexually compatible plant species as a result of gene transfer is **not an identified risk** and will not be assessed further.

Uncertainty

203. Currently there are limited incidences of sexually compatible species growing within the range of pollen flow from the proposed trial sites. There is also limited overlap of flowering times between current commercial varieties which may be used as the parent of the GM sugarcane plants and *S. spontaneum*. However, over the long term of this application the distribution of sexually compatible species may alter, or flowering times may change due to the introduction of new varieties of sugarcane, or changes in climatic conditions.

2.4 Horizontal transfer of genes or genetic elements to sexually incompatible organisms

204. Horizontal gene transfer (HGT) is the stable transfer of genetic material from one organism to another without reproduction (Keese 2008). All genes within an organism, including those introduced by gene technology, are capable of being transferred to another organism by HGT. HGT itself is not considered an adverse effect, but an event that may or may not lead to harm. A gene transferred through HGT could confer a novel trait to the recipient organism, through expression of the gene itself or the expression or mis-expression of endogenous genes. The novel trait may result in negative, neutral or positive effects.

205. Risks that might arise from horizontal gene transfer have been considered in previous RARMPs (eg DIR 057/2004 and DIR 085/2008), which are available from the OGTR website <http://www.ogtr.gov.au> or by contacting the Office. From the current scientific evidence, HGT from GM plants to other organisms presents negligible risks to human health and safety or the environment due to the rarity of such events, relative to those HGT events that occur in nature, and the limited chance of providing a selective advantage to the recipient organism.

206. Baseline information on the presence of the introduced or similar genetic elements is provided in Chapter 1, Section 6.5. Most of the introduced genetic elements are derived from naturally occurring organisms that are already present in the wider Australian environment.

Event 6. Presence of the introduced genetic material in other organisms as a result of horizontal gene transfer

207. Possible risks arising from HGT of the introduced genetic material to other organisms involves consideration of the potential recipient organism and the nature of the introduced genetic material. Risks that might arise from HGT from a GMO to another organism have been recently reviewed (Keese 2008) and considered in detail in a previous RARMP (DIR 085/2008) which is available from the OGTR website <http://www.ogtr.gov.au> or by contacting the Office.

208. HGT could result in the presence of the introduced genes or RNAi constructs for enhanced drought tolerance, enhanced nitrogen use efficiency, altered plant growth, altered sucrose accumulation or increased efficiency of post-harvest processing for cellulosic ethanol production in bacteria, plants, animals or other eukaryotes. However, the introduced sequences were mostly isolated from organisms already widespread in the environment (See Chapter 1, Section 6.5) and already available for transfer via demonstrated natural mechanisms.

209. A key consideration in the risk assessment process should be the safety of the protein product resulting from the expression of the introduced genes or RNAi constructs rather than horizontal gene transfer *per se* (Thomson 2000). If the introduced genes and RNAi constructs or their end products are not associated with any risk then even in the unlikely event of HGT occurring, they should not pose any risk to humans, animals or the environment. Conclusions reached for Events 1 - 4 associated with the expression of the introduced genes or RNAi constructs did not represent an identified risk. Therefore, any rare occurrence of HGT of introduced genetic material to other organisms is expected to be unlikely to persist and/or result in an adverse effect.

210. ***Conclusion:*** The potential for an adverse outcome as a result of horizontal gene transfer is **not an identified risk** and will not be assessed further.

2.5 Unintended changes in biochemistry, physiology or ecology

211. All methods of plant breeding can induce unanticipated changes in plants, including pleiotropy[11] (Haslberger 2003). Gene technology has the potential to cause unintended effects due to the process used to insert new genetic material or by producing a gene product that affects multiple traits. Such pleiotropic effects may include:

- altered expression of an unrelated gene at the site of insertion
- altered expression of an unrelated gene distant to the site of insertion, for example, due to the encoded protein of the introduced gene changing chromatin structure, affecting methylation patterns, or regulating signal transduction and transcription
- increased metabolic burden associated with high level expression of the introduced gene
- novel traits arising from interactions of the protein encoded by the introduced gene product with endogenous non-target molecules
- secondary effects arising from altered substrate or product levels in the biochemical pathway incorporating the protein encoded by the introduced gene.

[11] Pleiotropy is the effect of one particular gene on other genes to produce apparently unrelated, multiple phenotypic traits (Kahl 2001).

212. Such unintended pleiotropic effects might result in adverse outcomes such as toxicity or allergenicity, weediness, altered pest or disease burden, or reduced nutritional value as compared to the parent organism. However, accumulated experience with genetic modification of plants indicates that, as for conventional (non-GM) breeding programs, the process has little potential for unexpected outcomes that are not detected and eliminated during the early stage of selecting plants with new properties (Bradford et al. 2005).

Event 7. Changes to biochemistry, physiology or ecology of the GM sugarcane plants resulting from expression or random insertion of the introduced genes or RNAi constructs

213. No data is available on the phenotype of the GM sugarcane plants. Some phenotypic data is available for GM sugarcane containing some of the same genes or RNAi constructs released under DIR 070/2006 (refer to Chapter 1, Section 5.5.2 for details), however under the current release these genes and RNAi constructs would be combined with a greater variety of regulatory elements. Considerations relevant to altered biochemistry, physiology and ecology, in relation to expression of the introduced gene, have already been discussed in Events 1 to 3, and were not considered identified risks.

214. Various biochemical pathways of the GM sugarcane plants could be changed by the expression of the introduced genes or RNAi constructs, resulting in the production of novel or higher levels of endogenous toxins, allergens or anti-nutritional compounds.

215. Sugarcane products (from either GM or non-GM plants) can be detrimental if fed to animals in large quantities due to the presence of anti-nutritional compounds including hydrocyanic acid and lignin (Leng 1991; OGTR 2008b). Sugarcane pollen may also be an allergen (Chakraborty et al. 2001), although allergic responses to the commercial hybrid cultivars of sugarcane have not been reported in Australia. Further discussion regarding the toxicity and allergenicity of sugarcane is provided in *The Biology of the* Saccharum *spp. (sugarcane)* (OGTR 2008b).

216. Unintended secondary effects occurring as a result of enhanced drought tolerance, enhanced nitrogen use efficiency, altered plant growth, altered sucrose accumulation or increased efficiency of post-harvest processing for cellulosic ethanol production could include changes in plant growth, seed germination, seed dormancy, timing of flowering and seed set, outcrossing tendency or disease susceptibility (as discussed in Event 2). For example, in describing phenotypes of GM sugarcane lines released under DIR 070/2006, the applicant has noted unexpected effects on tillering and bud maturation in lines expressing *HvGA20ox-1* and *HvGA20ox -2*.

217. In plants, RNAi constructs can give rise to off-target silencing effects, where small RNAs derived from the sequence directing RNAi closely match non-target sequences expressed in the same cells. Homology of as little as 20 nucleotides can give rise to off-target silencing (reviewed by Small 2007). The strength of silencing of the non-target gene generally increases with greater lengths of homology and the strongest effects are expected to occur between highly homologous gene family members (Miki et al. 2005). Potential off-target silencing may be predicted if the sequence of the host genome is known, however this is not the case for sugarcane. Similarly to the effect of random insertions discussed below, any strong off-target silencing effect is likely to be detrimental to the plant.

218. The outcome of random insertion of an introduced gene is impossible to predict. Such outcomes may include, for example, alteration to reproductive capacity, altered capacity to deal with environmental stress, production of novel substances, and changes to levels of endogenous substances. Additionally, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland et al. 2003).

219. The likelihood of any pleiotropic effects causing adverse effects is minimised by the proposed limits and controls outlined in Chapter 1, Sections 3.2, and 3.3. In particular, the scale of the trial would limit the potential for adverse effects. Access to the proposed trial sites would be by private road, which limits exposure of the public to the GM plant material. Humans and livestock would not be intentionally exposed as the GM plant material will not be used as human food or animal feed.

220. ***Conclusion:*** The potential for an adverse outcome as a result of altered biochemistry, physiology or ecology is **not an identified risk** and will not be assessed further.

Uncertainty

221. As this is early stage research little is known about the GM sugarcane plants to be released. No primary screening would be carried out prior to planting at the trial sites. This assessment has been made based on the current context for the trial, so if this were to change over the fifteen year trial duration then there is uncertainty regarding the outcome of this assessment. Data regarding the phenotypes of the GM sugarcane lines would provide greater certainty to a future risk assessment.

2.6 Unintended presence of *Agrobacterium tumefaciens* containing the introduced genes or RNAi constructs, during release

222. *A. tumefaciens* is a soil-borne, Gram-negative bacterium that, in nature, causes crown gall in plants. The bacterium enters wounded cells of the host and causes surrounding host cells to proliferate irregularly and form a gall. The bacterium is confined to the cells of the gall. Eventually, degradation of the gall releases the *A. tumefaciens* back into the soil where it can live saprophytically for several years (Krimi et al. 2002; Escobar & Dandekar 2003).

223. For genetic modification, 'disarmed' strains of *A. tumefaciens* that cannot cause crown gall are used to transfer DNA to plant cells under controlled, optimized laboratory conditions. The strains used for genetic modification may also contain hypervirulent, attenuated tumour-inducing plasmids to increase cell transformation rates. *A. tumefaciens* has been shown to persist in *in vitro* plant tissues and shoots. Broad spectrum antibacterial compounds tend to have a bacteriostatic effect, suppressing, but not eliminating bacterial growth and when removed the bacteria may resume growth. In particular, Gram-negative bacteria (such as *A. tumefaciens*) are considered to be difficult to eradicate completely from *in vitro* cultures (Barrett et al. 1997; Leifert & Cassells 2001), although persistence of *A. tumefaciens* in some GM plants has not been detected (Charity & Klimaszewska 2005).

224. During *Agrobacterium*-mediated transformation of plant cells, the *A. tumefaciens* attaches to plant cell walls and a virulence system is activated in the bacterium, ultimately allowing the transfer and integration of bacterial DNA into the plant DNA (de la Riva et al. 1998). As with most bacterial endophytes, disarmed strains of *A. tumefaciens* would be expected to inhabit the intercellular spaces and xylem vessels of plant tissue (Rosenblueth & Martínez-Romero 2006) via the formation of surface-associated biofilms (Danhorn et al. 2008). This means it is highly unlikely that *A. tumefaciens* would be incorporated into plant reproductive cells. For this reason, *A. tumefaciens* may persist in vegetatively propagated GM plants (such as sugarcane) since there would be no opportunity for elimination of the *A. tumefaciens* in sexually produced generations.

225. The transfer of GM sugarcane plants, carrying *A. tumefaciens,* into the environment could result in the transfer of genes to non-target plants or other microorganisms (Leifert 2000). Possible risks associated with the use of *A. tumefaciens* for genetic modification of plants under laboratory conditions have also been considered in previous RARMPs concerning GM rose (DIR 060/2005), GM bananas (DIR 076/2007 and DIR 079/2007) and

GM torenia (DIR 084/2008). The RARMPs for these assessments are available at <http://www.ogtr.gov.au> or by contacting the OGTR.

Event 8. Transfer of the introduced genes or RNAi constructs from A. tumefaciens to other organisms

226. If *A. tumefaciens* containing an introduced gene construct was present in the GM sugarcane it could transfer the introduced genes or RNAi constructs via conjugation with a wild type strain or other bacteria and yeast naturally present in the soil at the site (Hammerschlag et al. 2000). This general possibility of horizontal gene transfer has already been discussed in Event 6 and was not considered to be an identified risk.

227. If the *A. tumefaciens* were present in GM sugarcane tissue it could also genetically modify cells of other plants. Although the conditions for *A. tumefaciens* infection and gene transfer to plants would exist in nature, the creation of a GM plant would be highly unlikely because it would be unlikely that the *A. tumefaciens* would genetically modify a cell or cells that would give rise to a new organism, and it is unlikely that conditions in nature would exist that would select for the survival of the infected GM plant cells.

228. The applicant proposes to generate the GM sugarcane plants using both biolistic and *Agrobacterium*-mediated genetic modification (Chapter 1, Section 5.4). The extent to which *Agrobacterium* persists in GM sugarcane is unclear, but is expected to be strongly reduced by inclusion of Timentin, and antibiotic acting against *Agrobacterium*, in tissue culture media. Arencibia et al. (1998) first reported *Agrobacterium*-mediated transformation of sugarcane, noting that *Agrobacterium* did not persist in lines characterised by Southern blotting. However, the transformation methods of Arencibia et al. differ from those described by the applicant. The applicant intends to PCR test each GM sugarcane plant for the presence of *Agrobacterium*, using primers specific to regions outside the T-DNA and would not release any plants positive for *Agrobacterium*. Given that the applicant proposes to transfer GM sugarcane plants directly from tissue culture to BSES stations for propagation on seedling benches, this measure is considered a necessary step to reduce the likelihood that plants transferred to the field would be carrying residual *A. tumefaciens*.

229. ***Conclusion:*** The potential for an adverse outcome resulting from the persistence in the environment of *A. tumefaciens* containing the introduced genes or RNAi constructs is **not an identified risk** and will not be assessed further.

2.7 Unauthorised activities

Event 9. Use of GMOs outside the proposed licence conditions (non-compliance)

230. If a licence were to be issued, non-compliance with the proposed conditions of the licence could lead to spread and persistence of the GM sugarcane plants outside of the proposed release areas. The adverse outcomes that this event could cause are discussed in the sections above. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires that the Regulator has regard for the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities.

231. ***Conclusion:*** The potential for an adverse outcome as a result of unauthorised activities is **not an identified risk** and will not be assessed further.

Section 3 Risk estimate process and assessment of significant risk

232. The risk assessment begins with a hazard identification process to consider what harm to the health and safety of people or the environment could arise during this release of GMOs

due to gene technology, and how it could happen, in comparison to the non-GM parent organism and in the context of the proposed receiving environment.

233. Nine events were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether, or not, expression of the introduced genes or RNAi constructs could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the spread and persistence of the GM plants; or produce unintended changes in their biochemistry or physiology. The opportunity for gene flow to other organisms and its effects if this occurred was also assessed.

234. A **risk** is only identified when a hazard is considered to have some chance of causing harm. Events that do not lead to an adverse outcome, or could not reasonably occur, do not represent an identified risk and do not advance any further in the risk assessment process.

235. The characterisation of the nine events in relation to both the magnitude and probability of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The principal reasons for this include:

- limits on the size and location of the release proposed by BSES
- suitability of controls proposed by BSES to restrict the dissemination and persistence of the GM sugarcane plants and their genetic material
- limited ability and opportunity for the GM sugarcane plants to transfer the introduced genes or RNAi constructs to commercial sugarcane crops or other sexually related species
- none of the GM plant materials or products will be used in human food or animal feed,
- widespread presence of most of the same proteins or sequences encoded by the introduced genes or RNAi constructs in the environment and lack of known toxicity or evidence of harm from them.

Therefore, any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM sugarcane plants into the environment are considered to be **negligible.** Hence, the Regulator considers that the dealings involved in this proposed release **do not pose a significant risk** to either people or the environment.

Section 4 Uncertainty

236. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communication. Both dimensions of risk (i.e. consequence and likelihood) are always uncertain to some degree.

237. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability[12]. For field trials, because they involve the conduct of research, some knowledge gaps are inevitable. This is one reason they are required to be conducted under specific limits and controls to restrict the spread and persistence of the GMOs and their genetic material in the environment, rather than necessarily to treat an identified risk.

238. For DIR 095 which involves early stage research, uncertainty exists in relation to the characterisation of:

[12] A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* (OGTR 2007) available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1> or via Free call 1800 181 030.

- Event 1, regarding potential increases in toxicity or allergenicity as a result of the introduced genes or RNAi constructs
- Event 2, associated with the potential for increased survival of the GMOs
- Event 3, associated with the potential for altered dispersal of the GMOs
- Event 4, associated with the potential for the expression of the introduced genes or RNAi constructs to alter the potential for sugarcane
- Event 5, associated with the potential for expression of the introduced genes or RNAi constructs to alter reproduction in the GM sugarcane
- Event 7, associated with the potential for any unintended effects as a result of changes in biochemistry, physiology or ecology of the GM sugarcane plants

239. Uncertainty associated with many of the events stems from potential changes in the risk context for the trial over the 15 year duration proposed by the applicant. This is further discussed in Chapter 3, Section 4.1.1.

240. Additional data, including information to address these uncertainties, would be required to assess possible future applications for a larger scale trial, reduced containment conditions, or the commercial release of these GM sugarcane plants if they are selected for further development.

241. Chapter 3, Section 5 discusses information that may be required for future releases.

Chapter 3 Risk management

242. Risk management includes evaluation of risks identified in Chapter 2 to determine whether or not specific treatments are required to mitigate harm to human health and safety, or the environment, that may arise from the proposed release. Other risk management considerations required under the Act are also addressed in this chapter. Together, these risk management measures are used to inform the decision-making process and determine licence conditions that may be imposed by the Regulator under the Act. In addition, the roles and responsibilities of other regulators under Australia's integrated regulatory framework for gene technology are explained.

Section 1 Background

243. Under section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment. All licences are required to be subject to three conditions prescribed in the Act.

244. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. Other mandatory statutory conditions contemplate the Regulator maintaining oversight of licensed dealings. For example, section 64 requires the licence holder to provide access to premises to OGTR monitors, and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

245. It is a further requirement that the licence be subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings and the possession, supply, use, transport or disposal of the GMOs for the purposes of, or in the course of, a dealing. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Responsibilities of other Australian regulators

246. Australia's gene technology regulatory system operates as part of an integrated legislative framework. Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, Therapeutic Goods Administration, National Health and Medical Research Council, National Industrial Chemicals Notification and Assessment Scheme and AQIS. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies[13].

247. The *Gene Technology Act 2000* requires the Regulator to consult these agencies during the assessment of DIR applications. *The Gene Technology (Consequential Amendments) Act 2000* requires the agencies to consult the Regulator for the purpose of making certain decisions regarding their assessments of products that are, or contain a product from, a GMO.

[13] More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator. Free call 1800 181 030 or at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

FSANZ is responsible for human food safety assessment, including GM food. As the trial involves early stage research, the applicant does not intend any material from the GM sugarcane plants proposed for release to be used in human food. Accordingly, the applicant has not applied to FSANZ to evaluate the GM sugarcane plants. However, in the event of a commercial release, FSANZ approval would need to be obtained before materials from the GM sugarcane plants could be sold for human consumption.

248. No other approvals are required.

Section 3 Risk treatment measures for identified risks

249. The risk assessment of events listed in Chapter 2 concluded that there are **negligible** risks to people and the environment from the proposed trial of GM sugarcane. The *Risk Analysis Framework* (OGTR 2007a), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation.

250. These events were considered in the context of the scale of the proposed release (a maximum total area of 21 ha on six BSES stations in Queensland over 15 years), the containment measures (Chapter 1, Section 3), and the receiving environment (Chapter 1, Section 6).

Section 4 General risk management

251. Licence conditions are imposed to control the dissemination and persistence of the GMOs and their genetic material in the environment and to limit the release to the size and locations requested by the applicant. Both of these considerations were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and environment are negligible. The conditions are detailed in the licence and summarised in Section 4.1.3.

4.1 Licence conditions

4.1.1 Consideration of limits proposed by BSES

252. Section 3.2 of Chapter 1 provides details of the limits proposed by BSES in their application, which are discussed in the events characterised for the release in Chapter 2. The appropriateness of these limits is considered further below.

253. The permitted dealings are confined to a maximum total area of 21 ha over six BSES stations, across five LGAs in Queensland. The applicant has proposed a fifteen year duration for this limited and controlled release. The previous releases of sugarcane under other DIR licences have been for much shorter terms, reflecting the 12-18 month cropping cycle of sugarcane. As discussed in Chapter 2 there is uncertainty relating to a number of the events arising from possible changes to the context of the trial over this time period. This may impact on the conclusions of the current risk assessment.

254. Agricultural practices may change leading to increased flowering of commercial cane or the introduction of varieties which flower in cooler regions. There is uncertainty surrounding the potential for gene flow in sugarcane as, until recently there was little research in this important area. As this research develops, this could alter the assessment of the potential for gene flow.

255. Over the longer term climate change may impact on the proposed release. It has been predicted that CO_2 levels are likely to double by 2050, with a 1.3-2˚C increase in temperature for Bundaberg (da Silva et al. 2008), suggesting that there would be a change in both CO_2 levels and temperature over the 15 year term of the licence. This could lead to altered

distribution of sexually compatible relatives, or altered flowering times of these plants, thus synchronising flowering of sexually compatible plants in the proposed trial sites where it is currently disparate. Experiments in which sugarcane was grown under double the current atmospheric level of CO_2 showed yield increases of 14% for the first harvest and 24% for ratoon crops (da Silva et al. 2008). The increase in the relative growth rates of crops from setts or from ratoons may lead to altered agricultural practices, with more ratooning than currently practised. The planting window for cane may also be altered with more planting in the winter period (SRDC 2007) and thus an altered flowering period. The increased temperature may also lead to an increase in pests and diseases, or the introduction of new pests to the region (SRDC 2007), which would necessitate altered agricultural practices, or the introduction of new varieties. Current predictions of the effects of climate change on Queensland include predictions of increased rainfall intensity and more intense tropical storms in some areas, particularly north Queensland (Queensland Government EPA 2008). If this eventuates then the proposed trial locations may become more likely to flood, which could increase the likelihood of dispersal of the GM sugarcane plant material.

256. This is an early stage field trial so very little is known about these GM lines and how they would behave under field conditions. It is strongly anticipated that within the 15 year time-scale proposed for the release there will be substantial advances in scientific understanding of the introduced genes and the genes targeted for silencing by the introduced RNAi constructs, particularly for those genes which are currently very poorly understood. In addition, further knowledge would come from evaluation of the GM sugarcane lines in the early stages of the proposed release (in field, clonal and progeny assessment trials of the GM sugarcane lines, before crossing is carried out). Such information would greatly reduce uncertainty by informing future risk assessments of the toxicity and allergenicity of the GM sugarcane (Event 1), the extent to which the genetic modifications may improve survival of the GM sugarcane lines (Event 2), the extent to which the GM sugarcane may disperse (Event 3) and cross with other sugarcane (Event 4) or sexually compatible species (Event 5), and unintended effects of the genetic modifications (Event 7).

257. Given the early stage nature of the proposed release, there are numerous areas of uncertainty including potential changes in the context within which risks have been assessed. Therefore, the imposed licence conditions limit the release to six years, rather than the 15 years proposed by the applicant.

4.1.2 Consideration of controls proposed by BSES

258. Section 3.3 of Chapter 1 provides details of the controls proposed by BSES in their application, which are discussed in the events characterised for the release in Chapter 2. The appropriateness of these controls is considered further below.

259. Only staff with appropriate training would be allowed access to the trial sites. Additionally, the applicant does not intend to use any of the GM plant material as human food or animal feed. These measures would limit the potential exposure of humans and vertebrates to the GMOs (Event 1) and the potential for the GM sugarcane plants to persist or to establish outside the proposed release sites (Event 3).

260. The trial sites would be located more than 50 m from the nearest natural waterways on land which has minimal risk of flooding (information provided by the applicant), which would reduce the likelihood of plant material being washed away from the sites (Event 3).

261. The applicant has proposed to test the GM sugarcane plants for *A. tumefaciens* and only release those that do not contain the bacterium. The extent to which *Agrobacterium* persists in GM sugarcane is unclear. The applicant proposes to transfer GM sugarcane plants from tissue culture directly to seedling benches at BSES stations on which field plantings would be

carried out. Given the large number of lines proposed for release and the short time between transformation and release, PCR testing is considered to be an important control to reduce the likelihood of *Agrobacterium*-mediated gene flow (Event 8), and so is included as an imposed licence condition.

262. There are no published studies of gene flow in sugarcane. Sugarcane pollen is thought to be produced in limited quantities and have low viability. Sugarcane has strong daylength and temperature requirements for flowering and viable pollen production, which effectively limit the production of significant amounts of viable seed to areas of Queensland north of the Burdekin river. The environmental conditions required for seed germination and establishment are also only found in north Queensland. The germination of seed and establishment of seedlings is limited by factors such as humidity, temperature, low intrinsic competitive ability, nutrient availability, pests and diseases and other environmental factors that normally limit the spread and persistence of sugarcane plants in Australia.

263. At the field sites, the applicant intends to surround the GM sugarcane with a guard row of non-GM sugarcane. The guard row would be separated from the nearest non-GM sugarcane by a 6 m isolation zone free of sexually compatible species. Such separation would help prevent inadvertent mixing of cane at harvest. In addition, the applicant has proposed further measures to reduce the likelihood of inadvertent harvesting of GM sugarcane, including marking field plantings with star pickets, signing GM plantings and informing and training staff on harvest and handling of GM sugarcane. Separation of GM from non-GM sugarcane with a guard row and isolation zone is considered an effective enough measure to reduce the likelihood of inadvertent harvesting of GM sugarcane, and so the further measures proposed by the applicant have not been imposed as licence conditions.

264. The applicant has proposed to cultivate GM sugarcane plants on seedling benches prior to planting in the field, using areas set aside at each BSES station at which field planting would occur. Non-GM sugarcane plants would also be cultivated in these facilities. The applicant proposes to keep GM and non-GM plants on separate benches, and barcode label the GM material. In order to reduce the potential dispersal of potted GM sugarcane plants by workers unaware of restrictions on their movement, the imposed licence conditions include a requirement to clearly label GM plants as being GM, and to sign benches containing GM material to indicate they hold GM plants.

265. In crossing facilities the applicant proposed to colour- and barcode label GM sugarcane and maintain GM sugarcane in clearly signed areas separate to areas used for non-GM sugarcane. These measures to reduce the potential for inadvertent mixture of GM and non-GM material have been included as licence requirements.

266. The applicant intends to contain flowering in those regions where environmental conditions are suitable for flowering, viable pollen production, seed germination and seedling survival (ie at the proposed crossing facilities at BSES Meringa and Southern). In crossing facilities the applicant proposes to separate GM and non-GM sugarcane, inspect GM sugarcane for signs of flowering three times per week in the period when the plants are likely to flower, and enclose GM sugarcane inflorescences in pollen lanterns prior to spikelet opening. This inspection frequency is considered appropriate for experienced sugarcane breeders to predict the onset of spikelet opening. The applicant describes the pollen lanterns as being impermeable to pollen, based upon the results of Skinner (1959). Skinner tested various materials for the ability to allow cross-pollination of an enclosed male sterile flower by nearby pollen-producing inflorescences. The applicant also proposes measures to control gene flow in the event that open spikelets are detected on the GM sugarcane before pollen lanterns are applied: open spikelets on the GM sugarcane would be removed and destroyed then the inflorescence enclosed in a pollen lantern, and nearby non-GM sugarcane would be

subject to the same measures to ensure no seed is set as a result of pollination outside pollen lanterns. These measures would reduce the potential for pollen flow (Events 4 and 5) and have been included as licence requirements.

267. The applicant has stated that any plant material taken off-site for experimental analysis and planting material moved between BSES stations would be transported according to the Regulator's Guidelines for the Transport of GMOs. These are standard protocols for the handling of GMOs to minimize exposure of humans and other organisms to the GMOs, dispersal into the environment, and gene flow/transfer. The applicant has proposed to transport sugarcane stems within BSES stations tied down in covered trailers. Sugarcane stems are considered propagative plant material, as they can potentially give rise to new sugarcane plants. The Regulator's transport guidelines require that viable or propagative GM material be enclosed by a primary and secondary unbreakable container when being transported, to minimise the risk of dispersal of GMOs. Displacement of stem pieces from trailers during transport could potentially lead to unintended establishment of GM sugarcane plants, thus increasing human and animal exposure to the GMOs (Event 1), allowing dispersal of the GM sugarcane (Event 2), and potentially enabling gene flow (Events 4 and 5). For these reasons, the requirement to transport all GM sugarcane material to and from trial sites according to the Regulator's Guidelines is imposed as a licence condition.

268. The applicant has proposed to destroy any viable GM plant material after final harvest by either burning, mulching or by herbicide treatment (Event 3). Similar disposal and/or destruction methods have been used previously for GM sugarcane under the licences for DIR 019/2002, DIR 051/2004 and DIR 070/2006 and are used in the industry as standard destruction methods (OGTR 2008b). This would minimise exposure to the GM plant material (Event 1) and limit the likelihood of spread and persistence (Event 2). However, it has also been proposed that stalks may be harvested and left to rot in the field, generally when these have been hand harvested. As discussed in Event 3 there is the potential for dispersal of viable cane pieces by animals and extreme weather conditions. Harvesting and leaving to rot in the field is not considered an acceptable method of destruction, and is not included as a condition in the licence.

269. The applicant has proposed to monitor the field release sites for 12 months post harvest and destroy any volunteer GM sugarcane, ensuring the sites are free of any volunteer sugarcane for a continuous period of at least six months. Sugarcane seed has little dormancy (Simpson 1990), and stem pieces capable of giving rise to new plants are not expected to remain viable over long periods, so this time period is considered appropriate for minimising the persistence of the GMOs in the environment (Event 2), potential for dispersal (Event 3) and gene flow (Events 4 and 5). A requirement to monitor the field release sites monthly for at least 12 months and until no volunteers have been detected for 6 months is included as a condition in the licence.

4.1.3 Summary of measures imposed by the Regulator to limit and control the release

270. A number of licence conditions have been imposed to limit and control the release, which are described in detail in the licence. These include requirements to:

- limit the release to six years
- surround the field trial sites with one guard row of non-GM sugarcane and a further isolation zone of at least 6 m
- separate GM sugarcane material from non-GM material when propagating seedlings or setts on seedling benches, and clearly identifying GM material

- separate GM from non-GM sugarcane in crossing facilities (glasshouses, pot holding areas, photoperiod glasshouses and crossing shed) and clearly identify GM material
- monitor GM sugarcane in photoperiod facilities for spikelet opening three times weekly and enclose inflorescences in pollen lanterns for controlled crossing, and destroy open spikelets not enclosed in pollen lanterns
- locate the field trial sites at least 50 m away from natural waterways
- harvest and process the GM sugarcane separately from any other sugarcane
- carry out analysis of plant materials only at the BSES stations or in PC2 laboratories
- destroy all plant materials not required for experimentation or propagation
- after cleaning of sites, monitor for and destroy any GM sugarcane that may grow for at least 12 months, and until no volunteers have been detected at the sites for a continuous 6 month period
- transport the GM plant materials in accordance with the Regulator's transportation guidelines
- not allow the GM plant material or products to be used for human food or animal feed.

4.1.4 Measures to control other activities associated with the trial

271. The Regulator has issued guidelines and policies for the transport and supply of GMOs (*Guidelines for the transport of GMOs; Policy on transport and supply of GMOs*). Licence conditions based on these guidelines and policies have been imposed regarding transportation and storage, and to control possession, use or disposal of the GMOs for the purposes of, or in the course of, the authorised dealings.

272. Conditions applying to the conduct of experimental analyses are also included in the licence conditions.

4.2 Other risk management considerations

273. All DIR licences issued by the Regulator contain a number of general conditions that relate to general risk management. These include, for example:

- applicant suitability
- contingency plans
- identification of the persons or classes of persons covered by the licence
- reporting structures, including a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to the health and safety of people or the environment
- a requirement that the applicant allows access to the trial sites by the Regulator, or persons authorised by the Regulator, for the purpose of monitoring or auditing.

4.2.1 Applicant suitability

274. In making a decision whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under section 58 of the Act matters that the Regulator must take into account include:

- any relevant convictions of the applicant (both individuals and the body corporate)
- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- the applicant's history of compliance with previous approved dealings
- the capacity of the applicant to meet the conditions of the licence.

275. On the basis of information submitted by the applicant and records held by the OGTR, the Regulator considers BSES suitable to hold a licence.

276. The licence conditions include a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability or their capacity to meet the conditions of the licence.

277. BSES must continue to have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

4.2.2 Contingency plans

278. BSES is required to submit a contingency plan to the Regulator within 30 days of the issue date of the licence. This plan would detail measures to be undertaken in the event of any unintended presence of the GM sugarcane lines outside of the permitted areas.

279. BSES is also required to provide a method to the Regulator for the reliable detection of the presence of the GMOs and the introduced genetic materials in a recipient organism. This instrument is required within 30 days of the issue date of the licence.

4.2.3 Identification of the persons or classes of persons covered by the licence

280. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence.

4.2.4 Reporting structures

281. The licence obliges the licence holder to immediately report any of the following to the Regulator:

- any additional information regarding risks to the health and safety of people or the environment associated with the trial
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the trial.

282. The licence holder is also obliged to submit an Annual Report within 90 days of the anniversary of the licence containing any information required by the licence, including the results of inspection activities.

283. A number of written notices are also be required under the licence that assist the OGTR in designing and implementing a monitoring program for all licensed dealings. The notices would include:

- expected and actual dates of planting
- expected and actual dates of commencement of flowering
- expected and actual dates of harvest and cleaning after harvest.

4.2.5 Monitoring for Compliance

284. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing. Post-release monitoring continues until the Regulator is satisfied that all the GMOs resulting from the authorised dealings have been removed from the release sites.

285. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

286. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. These include the provision for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to health and safety of people or the environment could result.

Section 5 Issues to be addressed for future releases

287. Additional information has been identified that may be required to assess an application for a large scale or commercial release of these GM sugarcane lines, or to justify a reduction in containment conditions. This would include:

- additional data on the potential allergenicity and toxicity of plant materials from the GM sugarcane lines
- phenotypic characterisation of the GM sugarcane lines, in particular of traits which may contribute to weediness, persistence, altered reproductive capability and ability to disperse in the environment
- molecular characterisation of the GM sugarcane lines
- additional information on potential pollen flow from sugarcane to sexually compatible species.

Section 6 Conclusions of the RARMP

288. The risk assessment concluded that this proposed limited and controlled release of up to 12,500 GM sugarcane lines on a maximum total area of 21 ha over 15 years in the Queensland LGAs of Moreton Bay, Bundaberg, Mackay, Burdekin and Cairns, poses **negligible** risks to the health and safety of people or the environment as a result of gene technology.

289. The risk management plan concluded that these **negligible** risks do not require specific risk treatment measures. However, licence conditions have been imposed to restrict the release to the size and locations requested by the applicant as these were important considerations in establishing the context for assessing the risks. The context for assessing the risks may change substantially over the 15 year period proposed by the applicant, potentially impacting upon the conclusions of the risk assessment. Therefore, the imposed licence conditions limit the release to six years, rather than the 15 years proposed by the applicant.

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Appendix A Definitions of terms in the *Risk Analysis Framework* used by the Regulator

(* terms defined as in Australia New Zealand Risk Management Standard AS/NZS 4360:2004)

Consequence
outcome or impact of an adverse event
Marginal: there is minimal negative impact
Minor: there is some negative impact
Major: the negative impact is severe

Event*
occurrence of a particular set of circumstances

Hazard*
source of potential harm

Hazard identification
the process of analysing hazards and the events that may give rise to harm

Intermediate
the negative impact is substantial

Likelihood
chance of something happening
Highly unlikely: may occur only in very rare circumstances
Unlikely: could occur in some circumstances
Likely: could occur in many circumstances
Highly likely: is expected to occur in most circumstances

Quality control
to check, audit, review and evaluate the progress of an activity, process or system on an ongoing basis to identify change from the performance level required or expected and opportunities for improvement

Risk
the chance of something happening that will have an undesired impact
Negligible: risk is insubstantial and there is no present need to invoke actions for mitigation
Low: risk is minimal but may invoke actions for mitigation beyond normal practices
Moderate: risk is of marked concern requiring mitigation actions demonstrated to be effective
High: risk is unacceptable unless actions for mitigation are highly feasible and effective

Risk analysis
the overall process of risk assessment, risk management and risk communication

Risk analysis framework
systematic application of legislation, policies, procedures and practices to analyse risks

Risk assessment
the overall process of hazard identification and risk estimation

Risk communication
the culture, processes and structures to communicate and consult with stakeholders about risks

Risk Context
parameters within which risk must be managed, including the scope and boundaries for the risk assessment and risk management process

Risk estimate
a measure of risk in terms of a combination of consequence and likelihood assessments

Risk evaluation
the process of determining risks that require treatment

Risk management
the overall process of risk evaluation, risk treatment and decision making to manage potential adverse impacts

Risk management plan
integrates risk evaluation and risk treatment with the decision making process

Risk treatment*
the process of selection and implementation of measures to reduce risk

Stakeholders*
those people and organisations who may affect, be affected by, or perceive themselves to be affected by a decision, activity or risk

States
includes all State governments, the Australian Capital Territory and the Northern Territory governments

Uncertainty
imperfect ability to assign a character state to a thing or process; a form or source of doubt

Appendix B Summary of issues raised in submissions received from prescribed experts, agencies and authorities[14] on the consultation RARMP for DIR 095

The Regulator received several submissions from prescribed experts, agencies and authorities on the consultation RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence that was used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence. A number of submissions received raised issues relating to risks to the health and safety of people and the environment as summarised below.

Summary of issues raised	Comments
Considers that there is potential for the genetic modifications to increase sugarcane's weediness, through the intended effects of genetic modifications to enhance nitrogen use efficiency, enhance drought tolerance, to alter plant growth, to improve sucrose accumulation and to improve cellulosic ethanol production, and also through unintended effects in all lines. States that no scientific data is provided to support the argument that changes to plant growth would not give rise to some selective advantages over non-GM sugarcane in the non-agricultural environment. States that consideration should be given to effects of WUE genes on both drought tolerance and water use efficiency, which could play an important role in the establishment of GM sugarcane from vegetative propagation. Drought tolerance plus water use efficiency may result in weediness.	The spread and persistence of sugarcane in the environment is limited by a complex range of factors including its low fertility and seed viability, nutrient requirements and susceptibility to pests and diseases. In the RARMP, the potential for the genetic modifications to increase the weediness of the GM sugarcane through intended and unintended effects is discussed (Event 2), and further discussion has now been introduced to the RARMP to provide additional general consideration of how weediness may be altered by the introduced traits. In the absence of phenotypic characterisation of the GM lines, the RARMP gives consideration to a wide range of reported effects of expression of the introduced genes in GM plants. It is noted that such phenotypes may not necessarily occur in the GM sugarcane, and that the discussion includes a much broader range of phenotypes than may actually occur. It is concluded that direct and unintended effects may incrementally increase weediness of the GM sugarcane. However, it is unlikely that the introduced genes or RNAi constructs would be able to alter all of the characteristics which limit the spread and persistence of sugarcane. In any case, measures have been imposed to restrict any potential spread and persistence of the GM sugarcane.

[14] GTTAC, State and Territory Governments, Australian Government agencies and the Minister for the Environment, Heritage & the Arts.

Summary of issues raised	Comments
Considers that the risk of gene transfer from GM sugarcane to non-GM sugarcane and other Saccharum species (especially *S. spontaneum*) exists. Recommends that at field sites, flowering should be monitored and inflorescences removed and destroyed if they are not required for further experimentation.	In Event 4, gene transfer from the GM sugarcane to other sugarcane and subsequent establishment of new plants is considered unlikely due to: • The infrequency of sugarcane flowering in southern regions of Queensland (eg BSES Woodford) • The very low levels of production of viable pollen in regions south of the Burdekin (eg BSES Woodford, and Southern) • Lack of known seedling survival in field conditions in central and southern Queensland (eg BSES Woodford, Southern, Central and Burdekin) • Poor seedling survival in field conditions in north Queensland (eg BSES Meringa). BSES Meringa is considered the only site at which conditions are such that all steps necessary for successful gene transfer could occur. At BSES Meringa all GM sugarcane inflorescences are required to be enclosed in pollen lanterns prior to anthesis, controlling the potential for pollen dispersal to any other sugarcane plants. In Event 5, the sexual compatibility of sugarcane with other species and the potential for crossing to occur is discussed. Other *Saccharum* species are not known to occur near any of the sites except BSES Meringa, where a clone garden containing several specimens is located, and *S. spontaneum* occurs in naturalised populations several kilometres away. Control measures have been imposed to restrict pollen dispersal from GM sugarcane at BSES Meringa.
States that the related genera *Vacoparis* and *Sarga* are found in sugarcane growing regions, and it is not known if sugarcane can hybridise to these native species though they are thought to be sexually compatible with sugarcane at very low levels (Bonnett et al 2008).	In Bonnett et al (2008) discussion of *Vacoparis* and *Sarga* is limited to noting that there are no reports on the ability of these genera to hybridise with sugarcane. However, as these species are closely related to *Sorghum*, their sexual compatibility with sugarcane may be similar. As discussed in the RARMP, hybridisation between sugarcane and *Sorghum bicolor* has been established to occur at very low frequency under artificial conditions and give rise to hybrids lacking vigour.
States that some sites are close to natural waterways, and occur in regions prone to flooding. 50 m separation from natural waterways is not satisfactory given the course of natural waterways, the length of the trial and the potential for flooding. Recommends a fence to trap floating plant material if relocating to a non-flooding zone is not practicable.	Further information on the likelihood of flooding at the proposed trial sites has been added to the RARMP. The trial sites were chosen, amongst other reasons, because "the risk of flooding is very minimal". The applicant has provided further information clarifying that BSES Meringa, Burdekin and Southern have no history of flooding, and BSES Woodford is in an elevated part of a flood-prone area and has experienced no significant flooding in the past 10 years. Flooding is rare at BSES Central, which is 5 km from the nearest major waterway, with the station being unaffected by significant floods in the area in 2008. The applicant also states that sugarcane plants are generally not uprooted by floodwaters, unless significant water flow occurs. Dispersal of GM sugarcane leading to its spread and persistence in the environment was therefore not considered an identified risk and control measures to restrict movement of pieces of GM material during flooding were not imposed.

Summary of issues raised	Comments
Considers that the release being conducted alongside ongoing work on non-GM sugarcane poses some level of risk of inadvertent mixing of GM and non-GM materials. Suggested controls included: • signing or fencing field plots to reduce the risk of inadvertent harvesting of GM sugarcane blocks • colour coding of pots to help differentiate GM and non-GM material in nurseries and crossing facilities, to reduce the potential for accidental dispersal of GM material • treating and destroying non-GM material in crossing facilities as if it were GM.	The proximity of the release to conventional sugarcane gave rise to consideration in the RARMP of the potential for GM sugarcane to pollinate non-GM sugarcane (Event 4), and also for inadvertent mixing of GM and non-GM sugarcane leading to dispersal of GM material (Event 3). In relation to the potential for accidental mixing of GM and non-GM sugarcane, the imposed licence conditions include controls to reduce the risk of this occurring, including: • physical separation of GM and non-GM sugarcane, clear labelling of GM material, and signing of areas in which GM material is kept. • Surrounding field plantings with a guard row and isolation zone 6 m wide to provide adequate physical separation from adjacent non-GM sugarcane. BSES has also indicated that they plan to implement a range of other measures to reduce the possibility of inadvertent harvesting of the GMOs, and BSES has not reported any problems relating to inadvertent harvest in their previous releases. • GM sugarcane in pots in nursery areas and crossing facilities must be physically separated from non-GM sugarcane, must be kept in areas signed as containing GM sugarcane, and must be clearly labelled. Colour coding of pots in nurseries and crossing facilities would be another way of easily distinguishing GM from non-GM sugarcane, and has been included in the licence as an example of the means by which GM and non-GM may be differentiated. The licence describes a crossing facility as being made up of several discrete areas of various parts of the BSES Meringa and Bundaberg stations (eg one area of a photoperiod facility, one area of a crossing shed, one area of a glasshouse). Non-GM material within areas designated for use with GM plants must be treated as if it were GM. In other parts of facilities within which GM plants are kept, non-GM material is not required to be destroyed since the licence requires separation measures as described above, and measures to restrict gene flow.
Considers that contingency plans should cover the possibility of cyclone damage, and should include treating any sugarcane material which falls within the 6 m isolation zone as if it were GM.	The licence requires that sugarcane or related species found within the isolation zone be destroyed. BSES is required by the licence to submit a contingency plan detailing measures to be undertaken in the event of inadvertent presence of the GM sugarcane lines outside the permitted areas.
Considers that the broad range of proposed geographic sites may increase the risk of spread and persistence in the environment.	The geographic distribution of the release sites is for the purpose of the lines being assessed under a range of environmental conditions. Importantly, the proposed areas of release are BSES experiment stations, at which managers have long-term experience in undertaking complex field trials and breeding work.

Appendix C Summary of issues raised in submissions received from the public on the consultation RARMP for DIR 095

The Regulator received two submissions from the public on the consultation RARMP. These submissions, summarised in the table below, raised issues relating to human health and safety and the environment. These were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Position (general tone): n = neutral; x = do not support; y = support

Issues raised: DR: data requirements; **EN**: environmental issues; **H**: human health; **HGT**: horizontal gene transfer; **OSA**: outside scope of assessment; **R**: regulatory process; **UE**: unintended effects.

Other abbreviations: GE: genetically engineered; **GM**: Genetically Modified; **GTR**: the Gene Technology Regulator; **RARMP**: Risk Assessment and Risk Management Plan.

Type: I: individual

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
1	I	x	UE	Objects to the release on the grounds of genetic instability.	Although the stability of the GM sugarcane lines for release has not been assessed, GM traits in sugarcane have been shown to be stably inherited, as is discussed in the RARMP. The spread and persistence of the GM sugarcane would be restricted by the limits and controls imposed upon this release.
			UE, EN	Objects to the release on the grounds of the unforseen ecological consequences of growing GE life forms in environments with which they are not harmonised.	DIR 095 is a limited and controlled release for the purpose of conducting experiments, including field characterisation of the GM sugarcane lines. The RARMP concluded that there were no identified risks to the environment from the GM sugarcane, and measures have been imposed to restrict their dissemination and persistence. The RARMP identifies information which may be required to assess future applications, such as phenotypic characterisation of the GM lines, including traits indicative of weediness. This information would be important in assessing the potential ecological consequences of larger scale or commercial releases of the GM sugarcane.
			H	Objects to the release on the grounds of the lack of social acceptance of GE foods, and unavoidability of consuming them once they are released. States that the threat of loss of natural sustenance is a considerable strain on human health and sense of well-being.	Material from the field trial is not allowed to be consumed by humans or animals. Social acceptance of GM food is outside the scope of the assessment conducted under the Gene Technology Act.

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
			OSA	Objects to the release on the grounds that climate change and the prospect of famine and disease have been caused by the impact of technology on the natural world, and by humans seeking to control nature.	Noted. The appropriateness of using gene technology is outside the scope of issues which the GTR must have regard to when deciding whether or not to issue a licence.
2	I	x	HGT, EN	Requests that DIR 095 exclude drought tolerance transgenic plants for reasons provided in relation to previous releases.	Please refer to comments addressing these submissions in the RARMPs for previous releases (DIRs 077/2007, 080/2007, 081/2007, 083/2007)
			HGT	Considers that evidence of problematic horizontal gene transfer continues to accumulate to the point that it would be negligent for the GTR to continue to claim that it provides no identifiable risk. Considers that the GTR must act to restrict release into the environment of genetically modified plants where transformation was achieved using *Agrobacterium* because of the increasingly realised and accepted higher risk of HGT. Given present knowledge it is unacceptable to allow release of GM plants produced by *Agrobacterium*-mediated transformation.	Potential risks to human health and safety and environment arising as a result of HGT were assessed in the RARMP. Important considerations were that: • the sequences are already widely present in the environment, and so are naturally available for HGT • assessment of the potential toxicity or allergenicity of the expressed proteins found no evidence that they posed a risk to human health and safety and the environment. Given the rarity of HGT occurring and that adverse consequence were found to be unlikely, it was concluded that the potential for an adverse outcome was not an identified risk. *Agrobacterium*-mediated transformation has been used over a considerable period of time, and adverse effects have not been detected.
			HGT	States that Ulker et al. (2008) confirm that use of the *Agrobacterium* transformation vector frequently (1:250) results in transfer and subsequent insertion into the host genome, of significant stretches (possibly 18 kb) of bacterial DNA (not necessarily from the Ti plasmid) linked to the transgene. Considers that the large *Agrobacterium* sequence linked to the T-DNA and incorporated into the host genome represents an homologous anchor for HGT from the plant to pathogenic *Agrobacterium* spp., related bacteria or as yet uncharacterised pathogenic bacteria, fungi, yeast and viruses. Considers that the 18 kb size of the anchor offers significant potential for a wide range of homologies, and it is obvious that a fragment of 18 kb will have some homology to DNA in many species	Ulker et al. (2008) found that 0.4% of *Arabidopsis* transformed by *Agrobacterium*-mediated transformation contained *Agrobacterium* chromosomal sequences, with sequences of over 18 kb being detected in linkage with T-DNAs. As the same mechanism of *Agrobacterium*-mediated gene transfer operates in sugarcane as in *Arabidopsis* these results are relevant to sugarcane, however the frequency of transfer of *Agrobacterium* chromosomal sequence in sugarcane is unknown. HGT to microorganisms is much more likely to occur from bacteria (such as *Agrobacterium*) than from plants (such as sugarcane). Plant genomes have been shown to contain sequences of bacterial origin, however these sequences have not given rise to HGT of plant genes to microorganisms. While presence of *Agrobacterium* sequences in GM plants could increase the frequency of HGT to species with homologous sequences, the most frequent outcome of this would be presence of *Agrobacterium* sequences in the HGT recipient organism. This outcome is already possible due to widespread presence of *Agrobacterium* in the environment. The basis for the assessment carried out in the RARMP is to consider hazards arising as a result of

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
				of micro-organisms. States that it is argued that HGT occurs at low frequency and so is not an identified risk, however considers that this is not true when homology is considered. Argues that HGT is well documented to occur at worrying frequencies (naturally and experimentally) and asks why these transformants have not been excluded from the licence?	release of the GM plants compared to what might naturally occur. For the current release the source organisms for the GM traits are generally widely persistent in the environment and so these sequences are already available for transfer. *Agrobacterium*-mediated transformation has been used over a considerable period of time, and adverse effects have not been detected.
			C	Why does the RARMP simply accept the applicant's claim that they will test for *Agrobacterium* with PCR? Considers that the applicant should identify the sequences they will test for, and what DNA will be tested. States that tests should be conducted to detect *Agrobacterium* DNA in the plant genome.	The RARMP assessed the dealings which BSES applied to conduct, which included PCR testing for the presence of *Agrobacterium*, and the licence conditions include PCR testing as a requirement. BSES provided the sequence of the PCR primers they plan to use to detect *Agrobacterium* in their application. Given that the presence of *Agrobacterium* DNA in the plant genome is not considered to give rise to an identified risk in relation to the potential for HGT, measures requiring it be tested for were not imposed.
			HGT	Considers that pathogenic *Agrobacterium* could transfer the traits acquired by HGT to other species which *Agrobacterium* can transform, which include plants, fungi, yeast and animal cells. States that the potential for this depends on where the traits insert in the bacterial genome, but it is likely that 1:250 such *Agrobacterium* would be capable of transforming plants with the cloned gene.	The chain of events necessary for the traits in the GM sugarcane to be transferred to *Agrobacterium* and then to other organisms includes: • generation of GM sugarcane plants in which the left and right border sequences of the T-DNA, which are not transferred intact during plant transformation, are reconstituted by chance events. • HGT of the entire gene and flanking border sequences from GM sugarcane to a strain of *Agrobacterium* capable of transforming plants, fungi and other species. Tumour-inducing plasmids carrying the genes necessary for T-DNA transfer occur only in a small percentage of natural *A. tumefaciens* populations in soil. • Contact between the recipient *A. tumefaciens* and other organisms in appropriate environmental conditions for transformation to occur. • For plants and animals, transformation of germ-line cells such that the received trait can be passed to offspring. While a chain of events giving rise to this outcome is plausible, there is a very small probability of each of these steps occurring, and so it is extremely unlikely for this entire chain of events to happen. Further, should all of these events occur, they would not necessarily result in harm.

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
			HGT, DR	Considers that the known potential *Agrobacterium* flanking sequences must be compared to other pathogens likely to be present in the soil at the release sites, which could readily be done as a database search. Argues that without this analysis, the potential for increased virulence of such pathogens due to transfer of stress tolerance genes cannot be ignored and the GTR is not in a position to claim no identified risk.	Risks to the health and safety of people or the environment that might arise as a result of HGT from the GM sugarcane to bacteria or fungi have been considered in the context of this limited and controlled release. The suggested analysis was not required in this assessment because consideration of the potential consequences concluded that it was unlikely that HGT would give rise to adverse consequences (see below). In addition, it is considered unlikely that HGT of the introduced genes from the GM sugarcane to microorganisms would occur. It is considered unlikely that HGT of the introduced genes from the GM sugarcane to microorganisms would occur and unlikely that such HGT would give rise to adverse consequences (see below). *Agrobacterium* occurs widely in the environment and so sequences from *Agrobacterium* are already available for horizontal gene transfer to other soil microorganisms.
			HGT, DR	States that the extent of expression of the cloned inserts (given their overexpression promoters and regulatory sequences) in bacteria, yeast and fungi needs to be examined or determined. Considers that the extent to which this would produce more virulent micro-organisms requires further speculation, and this possibility is high for stress tolerance transcription regulators (eg regulating osmosis or salt exclusion).	Risks to the health and safety of people or the environment that might arise as a result of HGT from the GM sugarcane to bacteria or fungi have been considered in the context of this limited and controlled release. The suggested analysis was not required in this assessment because consideration of the potential consequences concluded that it was unlikely that HGT would give rise to adverse consequences. It is considered highly unlikely that HGT of the introduced genes could produce drought tolerant bacteria or fungi. Given the vast differences in gene regulation between plants and bacteria or fungi, it is highly unlikely that genes conferring drought tolerance on plants would give rise to the same trait in bacteria or fungi. The eukaryotic promoters (which are not restricted to constitutive expression promoters) used in DIR 095 are unlikely to function similarly if transferred to bacteria or fungi. The encoded proteins and their products interact with a wide range of plant proteins which are not known to be present in bacteria or fungi.

Sub. No:	Type	Position	Issue	Summary of issues raised	Comment
			R	Considers genetic regulation is a joke and the GTR is redundant when releases of GM plants produced by *Agrobacterium* transformation is allowed, particularly when they have not been characterised properly or at all, and where the reason given for this is that PC2 facilities are not available.	The current assessment made use of published knowledge of effects of the genes of interest in other GM plants, published knowledge of the effects of GM plants conferred with similar traits, and data provided by the applicant relating to some of the genes of interest. The RARMP for this limited and controlled release concludes that risks to human health and the environment are negligible. The RARMP also identifies information which may be required to assess a larger-scale or commercial release, including phenotypic and molecular characterisation of the GM sugarcane lines.