Australian Government
Department of Health
Office of the Gene Technology Regulator

14 March 2019

Risk Assessment and Risk Management Plan (Consultation version) for

DIR 166

Limited and controlled release of *Cicer arietinum* (chickpea) genetically modified for drought and other environmental stress tolerance

Applicant: Queensland University of Technology

This RARMP is open for consultation until 29 April 2019.

Written comments on the risks to human health and safety and the environment posed by this proposed release are invited. You may make your submission

via mail to: The Office of the Gene Technology Regulator, MDP 54 GPO Box 9848, Canberra ACT 2601 or

via email to: ogtr@health.gov.au.

Please note that issues regarding food safety and labelling, the use of agricultural chemicals, and marketing and trade implications do **not** fall within the scope of these evaluations as they are the responsibilities of other agencies and authorities.

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Summary of the Risk Assessment and Risk Management Plan (Consultation version)

for

Licence Application No. DIR 166

Introduction

The Gene Technology Regulator (the Regulator) has received a licence application for the intentional release of a genetically modified organism (GMO) into the environment. It qualifies as a limited and controlled release application under the *Gene Technology Act 2000* (the Act). The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed field trial poses negligible risks to human health and safety and the environment. Licence conditions have been drafted for the proposed field trial. The Regulator invites submissions on the RARMP, including draft licence conditions, to inform the decision on whether or not to issue a licence.

The application

Application Number	DIR 166
Project Title	Limited and controlled release of *Cicer arietinum* (chickpea) genetically modified for drought and other environmental stress tolerance
Parent organism	Chickpea (*Cicer arietinum* L.)
Introduced genes	Introduced genes conferring drought and environmental stress tolerance: • *AtBAG4* – abiotic stress resistance gene from *Arabidopsis thaliana* • *TlBAG4* – abiotic stress resistance gene from *Tripogon loliiformis* Introduced marker gene: • *nptII* selectable marker - antibiotic resistance gene from *Escherichia coli*
Genetic modification method	*Agrobacterium*-mediated transformation
Number of lines	Up to 60 lines
Proposed location/s	Walkamin (Queensland Department of Agriculture and Fisheries Walkamin Research Facility), Tablelands Regional Council, Queensland
Proposed release size	Up to 3 ha per year
Proposed period of release	From July 2019 until December 2024
Principal purpose	To assess the drought and heat tolerance and agronomic characteristics of GM chickpea under field conditions

Risk assessment

The risk assessment concludes that risks to the health and safety of people or the environment from the proposed dealings are negligible. No specific risk treatment measures are required to manage these negligible risks.

The risk assessment process considers how the genetic modification and proposed activities conducted with the GMOs might lead to harm to people or the environment. Risks are characterised in relation to both the seriousness and likelihood of harm, taking into account current scientific/technical knowledge, information in the application (including proposed limits and controls) and relevant previous approvals. Both the short and long term impacts are considered.

Credible pathways to potential harm that were considered included exposure of people or other desirable organisms to the GM plant material, potential for persistence or dispersal of the GMOs, transfer of the introduced genetic material to non-GM chickpea plants. Potential harms associated with these pathways included toxicity or allergenicity to people, toxicity to desirable animals, and environmental harms due to weediness.

The principal reasons for the conclusion of negligible risks are that the GM plant material will not be used for human food or animal feed and that the proposed limits and controls will effectively minimise exposure to the GMOs.

Risk management

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions. Draft licence conditions are detailed in Chapter 4 of the RARMP.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the draft licence includes limits on the size, location and duration of the release, as well as controls to prohibit the use of GM plant material in human food and animal feed, to minimise dispersal of the GMOs or GM pollen from the trial site, to transport GMOs in accordance with the Regulator's guidelines, to destroy GMOs at the end of the trial and to conduct post-harvest monitoring at the trial site to ensure all GMOs are destroyed.

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Abbreviations

Act	*Gene Technology Act 2000*
AMF	Arbuscular mycorrhizal fungi
APVMA	Australian Pesticides and Veterinary Medicines Authority
Bag	*Bcl-2*-associated athanogene
BAG	Protein expressed by *Bag gene*
Bag4	Bcl-2-associated athanogene 4
BAG4	Protein expressed by *Bag4* gene
Bcl-2	B-cell lymphoma-2 proteins
BD	BAG domain
CaMV	Cauliflower mosaic virus
CaMV35S	35S RNA promoter from CaMV
CCIA	California Crop Improvement Association
CSGA	Canadian Seed Growers' Association
DIR	Dealings involving Intentional Release
DNA	deoxyribonucleic acid
EST	Expressed sequence tag
FAOStat	Statistics Division, Food and Agriculture Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
ft	Feet
GM	genetically modified
GMO	genetically modified organism
Ha	Hectare
HR	Hypersensitive response
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
LGA	Local Government Area
m	metres
NLRD	Notifiable Low Risk Dealing
nptII	Neomycin phosphotransferase II gene
NSW	New South Wales
NSW DPI	NSW Department of Primary Industries
OGTR	Office of the Gene Technology Regulator
PC2	Physical Containment level 2
PCD	Programmed cell death
QDAF	Queensland Department of Agriculture and Fisheries
Qld	Queensland
QUT	Queensland University of Technology
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
SA	South Australia
SCSV	Subterranean clover stunt virus
SCSV pS1	Promoter from SCSV DNA segment 1
USDA	United States Department of Agriculture
USDA-APHIS	United States Department of Agriculture Animal and Plant Health Inspection Service
Vic.	Victoria
WA	Western Australia
WHO	World Health Organization
WT	Wild type

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under *the Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.

2. The Act and the Gene Technology Regulations 2001 (the Regulations), together with corresponding State and Territory legislation, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.

3. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.

4. The Risk Analysis Framework (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator (OGTR) website.

5. Figure 1 shows the information that is considered, within the regulatory framework, in establishing the risk assessment context. This information is specific for each application. Potential risks to the health and safety of people or the environment posed by the proposed release are assessed within this context. Chapter 1 provides the specific information for establishing the risk assessment context for this application.

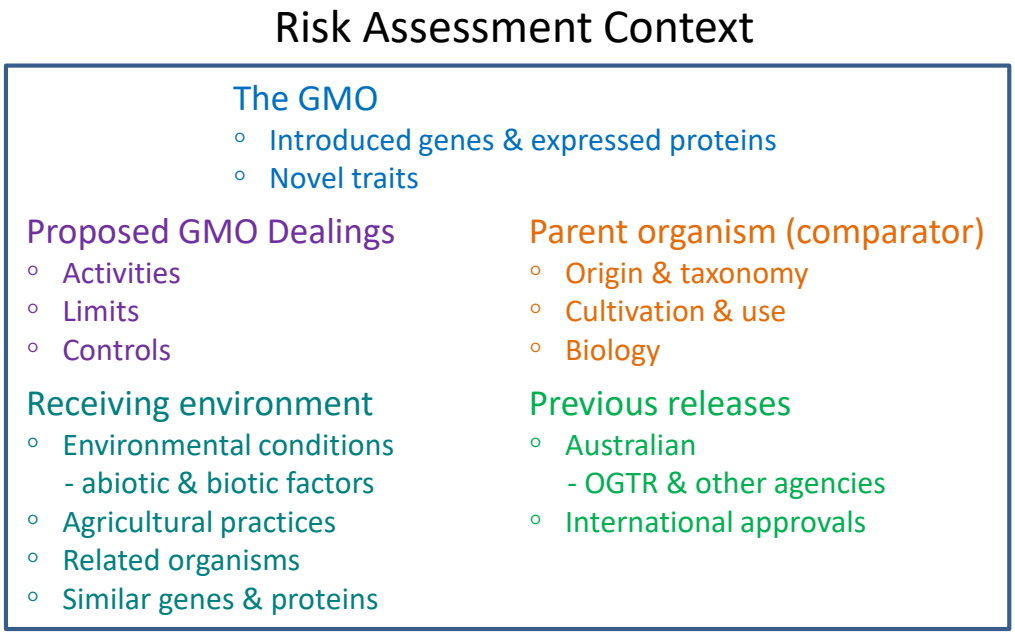

Figure 1 Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.

6. In accordance with section 50A of the Act, this application is considered to be a limited and controlled release application, as the Regulator was satisfied that it meets the criteria prescribed by the Act. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

7. The GMOs and any proposed dealings may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority, the Therapeutic Goods Administration and the Department of Agriculture and Water Resources. Proposed dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

Section 2 **The proposed dealings**

8. Queensland University of Technology (QUT) proposes to release up to 60 lines of chickpeas genetically modified for drought and environmental stress tolerance. The purpose of the release is to evaluate the drought and heat tolerance and agronomic performance of GM chickpea lines under field conditions.

9. The dealings involved in the proposed intentional release are:

- conducting experiments with the GMOs
- propagating the GMOs
- growing the GMOs
- transporting the GMOs
- disposing of the GMOs

and possession, supply or use of the GMOs for any of the purposes above.

2.1 The proposed limits of the dealings (duration, size, location and people)

10. The release is proposed to take place for up to six growing seasons, from the issue of the licence until December 2024. GM chickpea would be grown on a single trial site with an area of up to 3 ha per season. The trial site would be located at the Queensland Department of Agriculture and Fisheries (QDAF) research station at Walkamin, Queensland, approximately 70 km west of Cairns.

11. Only trained and authorised staff would be permitted to deal with the GM chickpea.

2.2 The proposed controls to restrict the spread and persistence of the GMOs in the environment

12. The applicant has proposed a number of controls to restrict the spread and persistence of the GM chickpea and the introduced genetic material in the environment. These include:

- locating the proposed trial sites at least 100 m away from the nearest natural waterway, in areas not prone to flooding
- surrounding the trial site with a 3 m monitoring zone and a 5 m isolation zone in which no chickpeas will be intentionally planted
- only permitting trained and authorised staff to access the trial site
- controlling rodents at the trial site
- restricting animal access by surrounding the trial site with fences
- treating non-GM plants used in the trial as if they were GM
- inspecting all equipment after use for GM seeds and cleaning as required
- transporting and storing GM plant material in accordance with the current Regulator's Guidelines for the Transport, Storage and Disposal of GMOs
- destroying all plant material from the trial not required for testing or future trials
- post-harvest monitoring of the trial site at least once every two months for at least 18 months and until the site is free of volunteer plants for six months, with any chickpea volunteers destroyed prior to flowering
- one shallow tillage postharvest when conditions are conducive to germination of volunteers
- the site would be irrigated postharvest, if required, to promote germination of volunteers
- not allowing the GM plant materials or products to be used in commercial human food or animal feed

Section 3 **The parent organism**

13. The parent organism is *Cicer arietinum* L. (chickpea). Detailed information about chickpea is contained in the reference document *The Biology of* Cicer arietinum *L. (chickpea)* (OGTR, 2019), which was produced to inform the risk analysis for licence applications involving GM chickpea. Baseline information from this document will be used and referred to throughout the RARMP.

14. Chickpeas are grown in Queensland (Qld), New South Wales (NSW), Victoria (Vic.), South Australia (SA) and Western Australia (WA) (ABARES, 2018). Chickpea cropping areas are divided into five regions: Region 1: tropical, low rainfall (central Qld); Regions 2 and 3 sub-tropical, medium and low rainfall respectively (northern NSW & southern Qld); Regions 4 and 5, Mediterranean medium/high and low/medium rainfall respectively (southern NSW, north western Vic, south eastern SA and south western WA) (Pulse Australia, 2016).

15. The majority of the Australian crop is produced in Qld and NSW, with five year production averages to 2017-18 of 444,000 t and 531,000 t respectively, with smaller production in Vic. (39,000 t), SA (22,000 t) and WA (6,000 t) (ABARES, 2018). Areas planted and volume of production are forecast to decline sharply in 2018-19 in Qld and NSW due to reduced export demand and seasonal conditions (ABARES, 2018). Total annual production during the five seasons from 2012 to 2016 varied from 555,440 t (2015) to 874,593 t (2016), with annual exports of 550,567 t to 1,286,718 t over the same period (export does not necessarily occur in the same production year) and a value of $US 295 million - $US 906 million (FAOStat, 2018). Since Australia commenced trading internationally in 1988 it has been among the top five exporters each year and has been the largest exporter of chickpeas from 2008 – 2016 (FAOStat, 2018).

16. Two types of chickpeas are grown in Australia, over 90 % of production as Desi chickpeas and up to 10 % as Kabuli chickpeas. Desi chickpeas have smaller angular seeds with a wrinkled beak, with different varieties of varying colours. The seeds are usually dehulled and split to obtain dhal, or may be used to produce besan flour, although some larger varieties are used whole. Kabuli chickpeas, also known as garbanzos, have larger more rounded seeds and are white or cream in colour and are used whole (Pulse Australia, 2016). An indication of the variation in seed size, shape and colour is shown in *The Biology of* Cicer arietinum *L. (chickpea)* (OGTR, 2019 and references therein)

17. Chickpeas are grown as a winter crop in Australia, planted between late April and mid July, with preferred planting time determined by region, rainfall, disease conditions and disease resistance of the varieties being planted. Within each chickpea growing area harvest may occur over a period of 4 – 6 weeks for crops planted at the same time, with resulting differences in crop moisture content. Early harvest can be targeted by a number of means including early sowing where possible, variety selection and planting standards, disease and insect control, desiccation of crops before harvest and harvest conditions. Late harvest can result in reduced yield and quality. While chickpea planting times provide an opportunity outside planting times for winter cereals, chickpea harvest can clash with wheat harvest and growers must make decisions for harvest based on crop quality and potential returns (Pulse Australia, 2015, 2016; GRDC, 2018).

18. Chickpeas are primarily a food crop that has been consumed by humans for many centuries, as well as being used for traditional medicines and cosmetics (OGTR, 2019 and references therein). Consumption in Turkey and India is over 5 kg per capita annually (Yadav et al., 2007). The chickpea plant does not produce acute toxins and its components are not considered to be toxic, although they do produce irritants and antinutritional factors. Chickpea leaves secrete acids from leaves, stems and pods (van der Maesen, 1972; Khanna-Chopra and Sinha, 1987; Narayanamma et al., 2013; GRDC, 2017b) that are irritants which affect the skin eyes and respiratory tract of humans (NCBI, 2019), however malonate secreted by the roots is degraded by microorganisms and does not accumulate in the soil (Wouterlood et al., 2004). A number of antinutritional factors are present in chickpeas (Williams and Singh, 1987; Alajaji and El-Adawy, 2006; Muzquiz and Wood, 2007), including protease inhibitors (trypsin, chymotrypsin), low levels of phytohaemagglutinins, phytic acids that can bind essential minerals, polyphenols including tannins and trace levels of cyanogenic glycosides. They also contain oligosaccharides that are undesirable as flatulence factors, but are a source of fibre and a prebiotic food source for gut bacteria, as well as saponins that can

interfere with nutrient uptake but may also reduce cholesterol levels (OGTR, 2019). The majority of these components are reduced by cooking and processing in food preparation (Muzquiz and Wood, 2007; Bampidis and Christodoulou, 2011). Mycotoxins may also be associated with stored chickpeas.

19. Chickpea allergies have been recorded (Patil et al., 2001; Martínez San Ireneo et al., 2008), particularly in countries where consumption of chickpeas is high and/or in individuals who are allergic to other legumes or tree nuts (Barnett et al., 1987; Patil et al., 2001; Martínez San Ireneo et al., 2008; Bar-El Dadon et al., 2014). No chickpea allergens are registered in the WHO/IUIS[1] Allergen Nomenclature database (WHO/IUIS Allergen Nomenclature Sub-Committee, 2018); however putative allergens have been identified that are related to allergens found in other legumes (Bar-El Dadon et al., 2014).

20. Chickpeas are mainly self-pollinating with pollination occurring 1 -2 days before flowers open fully (van der Maesen, 1972; Kalve and Tadege, 2017). Although insects visit open chickpea flowers (van der Maesen, 1972; Tayyar et al., 1996), there is no evidence of insect or animal pollination increasing seed production in chickpeas (Klein et al., 2007). Recorded outcrossing rates in overseas trials in close-planted chickpeas are very low, from zero to 4.2 %, with averages below 2 % (Niknejad and Khosh-Khui, 1972; Gowda, 1981; Malhotra and Singh, 1986; van Rheenen et al., 1990; Tayyar et al., 1996; Toker et al., 2006). A higher rate of 5.9 % recorded only when an open-flowered mutant was used (Srinivasan and Gaur, 2012). No information is available on intraspecific outcrossing for chickpeas in Australia.

21. Natural interspecific crossing is unlikely in chickpea and has not been reported, as plants in genus *Cicer* are almost entirely self-pollinating (van der Maesen, 1987). In addition, the two species which form the primary gene pool for chickpea, *Cicer reticulatum* and *Cicer echinospermum*, occur only in Turkey and Iraq (Croser et al., 2003; van der Maesen et al., 2007) and are not present in Australia. Neither species is listed as weedy (Randall, 2017) and *C. reticulatum* is listed by the International Union for Conservation of Nature as "Near Threatened", while *C. echinospermum* is not classed as threatened (IUCN Red List database, accessed 5 February 2019). *C. reticulatum* is generally found in rocky areas, while *C. echinospermum* may be found growing as a weed in cultivated areas (Abbo et al., 2007). Neither species is cultivated.

22. While some species of *Cicer* exhibit varying levels of innate seed dormancy (Singh and Ocampo, 1997), the ancestor of modern chickpeas, *C. reticulatum*, does not tend to show seed dormancy, so unlike many crop species, this was not a focus of domestication (Abbo et al., 2003). There is no evidence for dormancy in chickpea cultivars (MoEF&CC, 2016; OGTR, 2019).

23. A weed risk assessment for chickpeas has been prepared (OGTR, 2019). Briefly, chickpea lacks many common weedy characteristics. It has been grown globally for centuries, without any reports that it has become a serious weed. Chickpea is regarded as a category 1[2] weed of natural ecosystems and as a naturalised weed of agricultural ecosystems in Australia, with a category 1 classification in SA, so it is not considered that control is warranted at any location (Groves et al., 2003). On a global scale, chickpeas are not currently regarded as a weed and are a low weed risk (Randall, 2017). Weedy populations are not found in natural ecosystems, nor in areas such as roadsides along transport routes (OGTR, 2019), with few specimens collected outside cultivation in Australia (Atlas of Living Australia, 2018).

[1] World Health Organization and International Union of Immunological Societies

[2] Category 1 weeds are characterised as naturalised and may be a minor problem but not considered important enough to warrant control at any location.

Section 4 The GMOs, nature and effect of the genetic modification

4.1 Introduction to the GMOs

24. The GM chickpeas proposed for release contain one of two genes for abiotic stress tolerance, with up to 30 lines proposed for each gene. As each line will contain only a single inserted gene, the applicant proposes to release up to 60 lines of chickpeas.

25. The GM chickpea lines were/will be produced using *Agrobacterium*-mediated transformation. Information about the *Agrobacterium*-mediated transformation method can be found in the document *Methods of plant genetic modification* available from the OGTR Risk Assessment References page. Additionally, the applicant has stated that they routinely test for *Agrobacterium* in transformed plants and select only those that test negative to use for further work.

26. The introduced genes are derived from *Arabidopsis thaliana*, a small plant commonly used as a model organism in plant biology and from *Tripogon loliiformis*, an Australian grass from arid northern regions. Genes and regulatory elements introduced to GM chickpea lines are shown in Table 1.

Table 1: Genes and regulatory elements introduced to GM chickpea lines

Genetic element	Gene Source	Description	Function
AtBag4	*Arabidopsis thaliana*	Open reading frame of *Bcl-2*[a]-associated athanogene 4	Abiotic stress tolerance
TlBag4	*Tripogon loliiformis*	Open reading frame of *Bcl-2*-associated athanogene 4	Abiotic stress tolerance
35S	Cauliflower mosaic virus	Promoter from CaMV[b]	Promoter for *BAG4* genes
pS1	Subterranean clover stunt virus	Promoter from SCSV[c]	Promoter for marker gene
nptII	*Escherichia coli*	Plasmid selectable marker - kanamycin	Selectable marker gene
tNos	*Agrobacterium tumefaciens*	Terminator and polyadenylation signal of the nopaline synthase gene	Terminator sequence

[a] Bcl-2: B-cell lymphoma-2 proteins
[b] CaMV: Cauliflower mosaic virus
[c] SCSV: Subterranean clover stunt virus

27. Short regulatory sequences that control expression of the genes are also present in the GM chickpea lines (Table 1). The regulatory sequences are derived from microorganisms (Cauliflower mosaic virus (CaMV), Subterrranean clover stunt virus (SCSV) or *Agrobacterium tumefaciens*).

28. The GM chickpea plants also contain the *nptII* (neomycin phosphototransferase II) selectable marker gene (Table 2). Selectable markers are used in the laboratory to select transformed GM plants or plasmids during early stages of development. This gene is derived from *Escherichia coli* (*E. coli*) strain K12 and encodes an aminoglycoside 3'-phosphotransferase II enzyme that is also known as neomycin phosphototransferase II (NPTII). It provides resistance to kanamycin and related antibiotics. More information on marker genes is available in the document Marker Genes in GM Plants.

4.2 The introduced genes, encoded proteins and associated effects

4.2.1 Introduction to Programmed Cell Death

29. The term programmed cell death (PCD) is used to describe the process(es) of organised and controlled destruction of cells. It is conserved across broad evolutionary distances and is critical to development, homeostasis and responses to biotic and abiotic stresses (Doukhanina et al., 2006; Kabbage et al., 2017). The types of cell death occurring as part of PCD are a continuum of processes ranging from highly organised apoptosis, to autophagy, to tissue necrosis which is generally regarded as less controlled and organised (Williams and Dickman, 2008; Dickman et al., 2017).

30. In plants, the processes and controls for PCD are less well understood than those for animals (Doukhanina et al., 2006) and some researchers argue that plants do not have apoptosis or apoptosis-like processes due to fundamental differences between plant and animal cells (van Doorn, 2011; van Doorn et al., 2011). However, it is likely that plants, like animals, use a number of regulatory pathways to control cell death (Dickman et al., 2017 and references cited therein) and that they undergo apoptosis-like[3] processes in response to biotic and abiotic stresses (Curtis and Wolpert, 2004; Doukhanina et al., 2006; Hoang et al., 2015; Kabbage et al., 2016; Dickman et al., 2017; Kabbage et al., 2017). Although apoptosis in plants is not yet fully understood, a number of apoptotic features similar to those understood for mammalian systems are present in plants. These include cell shrinkage, chromatin condensation, phosphatidylserine externalisation, DNA laddering, characteristic DNA cleavage, involvement of capsases or protease cell death signalling, permeability and depolarisation of mitochondria, cytochrome c release, formation of apoptotic bodies (Dickman et al., 2017 and references cited therein).

4.2.2 The introduced genes

31. The genes and their encoded proteins are summarised in Table 1, with a description of their potential function in the GM chickpea lines. Both introduced genes are from the *Bcl*-2-associated athanogene (*Bag*) group of genes. These genes encode BAG proteins, which are a group of proteins that are evolutionarily conserved across a wide variety of organisms (Doukhanina et al., 2006; Kabbage et al., 2017). BAG proteins regulate diverse physiological processes in animals, including apoptosis, tumorigenesis, neuronal differentiation, stress responses, and the cell cycle (Doukhanina et al., 2006). It has been suggested that sequence homology between animal and plant genomes is not a good predictor of function for this group of proteins and that protein structural similarities, particularly in functional regions may provide an explanation for 'operational equivalence' independent of sequence homology (Dickman et al., 2017).

32. The BAG proteins contain a common Hsp70/Hsc70 interaction domain, the BAG domain (BD), but differ in the N-terminal region which is related to specificity to particular proteins or pathways (Doukhanina et al., 2006). The *AtBag4* gene is one of seven *Bag* genes characterised from *A. thaliana*, four of which - including BAG4 - have similar domain structure to human BAG1 proteins, with a ubiquitin-like motif in addition to the BD, while the other three have a calmodulin-binding motif near the BD. The calmodulin-binding motif is unique to plant BAG proteins (Kabbage et al., 2017). At least three of the *Arabidopsis* BAG proteins – BAG4, BAG6 and BAG7 – have confirmed cytoprotective roles in responses to cold, drought and heat (Doukhanina et al., 2006; Kabbage and Dickman, 2008; Kabbage et al., 2017).

33. The AtBAG4 protein expressed by the *AtBag4* gene has high structural homology to human BAG4 protein, particularly in functionally important domains (Doukhanina et al., 2006; Kabbage and Dickman, 2008). The AtBAG4 protein is involved in inhibiting cell death in response to abiotic factors such as UV, oxidants, salt, drought and cold stress (Doukhanina et al., 2006; Kabbage et al., 2017). In GM tobacco plants overexpressing the *AtBag4* gene, the level of expression (low medium or high) can influence the phenotype, with, for example, resistance to UV light exposure inversely proportional to expression levels (Doukhanina et al., 2006). Additionally, when wild type (WT) tobacco plants and tobacco lines with low-level expression of the *AtBag4* gene were examined for markers of apoptosis following exposure to cold stress, apoptosis markers were present in the WT plants, but were not detected in *AtBag4*-expressing lines (Doukhanina et al., 2006).

34. Database searches have indicated that BAG-like genes are widely distributed across plant genomes, with expressed sequence tags (ESTs) in a range of species, either in specific tissues (root, flower, inflorescence), or in plant tissues subjected to biotic and abiotic stresses (Doukhanina et al., 2006). These findings suggest that BAG protein expression is involved in developmental and environmental responses (Doukhanina et al., 2006; Kabbage and Dickman, 2008). An EST match was found for a chickpea *Bag*-like

[3] The term 'apoptosis-like' is used in relation to programmed cell death in plants, due to ongoing discussion about whether apoptosis, as understood for animal cells, occurs in plants. However, for clarity, the term apoptosis is used in this document.

gene (Doukhanina et al., 2006) and phylogenetic trees have grouped a *Bag*-like gene from chickpea with other plant *Bag* genes (Doukhanina et al., 2006; Rana et al., 2012).

35. Overexpression of the *AtBag4* gene in GM rice conferred salinity tolerance (Hoang et al., 2015). In other studies, rice *Bag* genes have been shown to be up- or down-regulated during different types of stress (biotic or abiotic) and were upregulated in plants during the early stages of heat stress, with expression declining as the stress continued, indicating roles in early responses to heat stress (Rana et al., 2012).

36. Mammalian BAG proteins bind with heat shock protein 70 (HSP70) and heat shock cognate (HSC70) chaperones (Doukhanina et al., 2006). Although the biochemical details of the activity of the BAG4 protein in preventing PCD in plants are still unclear (Kabbage et al., 2017), an association between AtBAG4 protein and HSC70s has been established (Doukhanina et al., 2006).

37. The *TlBag4* gene is a homologue of the *AtBag4* gene, identified from a de novo assembled transcriptome. It is expected that overexpression of this gene in GM chickpea will increase drought and heat tolerance in the field through the stress tolerance function of this gene (information provided by the applicant).

4.2.3 Source organisms for the genes

38. The source organism *A. thaliana* (thale cress or mouse ear cress) is a small herbaceous annual flowering plant in the Brassicaceae family (mustard family, which also includes cabbage and broccoli). It is regarded as the most widely used model organism in plant biology (Koornneef and Meinke, 2010) due to small genome size, chromosome number, fast growth cycle, small plant size, autogamous breeding system and ability to grow on various synthetic media (Flora of North America, accessed 21 January 2019). Although edible, it is not generally used as food (Atlas of Living Australia; accessed January 21 2019). It is generally considered a weed, due to its widespread distribution in agricultural fields, roadsides, and disturbed lands. *Arabidopsis thaliana* is naturalised in Australia (Atlas of Living Australia; accessed 21 January 2019), but it is not listed as a weed of national significance (Weeds of National Significance, accessed 21 January 2019). It has been listed as low weed risk based on its pattern of entry, means of dispersal and impact (Randall, 2016; Randall, 2017). This plant has been the source of genes and or regulatory elements in a wide range of GM plants, with no reported adverse effects.

39. The source for the *TlBag4* gene, *T. loliiformis* is a small grass native to Australia and New Guinea which grows as a locally abundant species in very specific habitats, often in shallow soils close to rocky outcrops, as colonisers of shallow soil, especially where water is limited (Gaff and Latz, 1978; Scharaschkin and Fabillo, 2015). In such areas they colonise and trap further soil and detritus, then other species begin to colonise and overshadow *T. loliiformis* (Gaff and Oliver, 2013). It is also sensitive to disturbance and will not survive in habitats that are subject to disturbance even if conditions are otherwise suitable (Scharaschkin and Fabillo, 2015). This species is not weedy (Weeds of National Significance, accessed 21 January 2019; (Randall, 2017) and in Victoria is listed as “Near Threatened” (Atlas of Living Australia, accessed 21 January 2019).

40. Known as a ‘resurrection plant’ *T. loliiformis* is a species with the ability to tolerate desiccation, which is apparent in individuals occurring in a range of habitats and with varying morphological forms (Scharaschkin and Fabillo, 2015). Plants can survive periods of water deficit, reviving when water is available, and they can potentially undergo a number of cycles of desiccation and revival (Gaff and Oliver, 2013), although it is not known how long they can survive in the desiccated state and revive (Scharaschkin and Fabillo, 2015). It is apparent that rather than ‘resurrecting’, *T. loliiformis* plants maintain viability and revive after periods of desiccation, although the exact mechanisms of how this occurs are not yet understood. Experimental results indicate that autophagy is triggered during desiccation, which is part of survival mechanisms that supress PCD and senescence (Williams et al., 2015).

4.3 Toxicity/allergenicity of the proteins associated with the introduced genes

41. As the GMOs are at an early stage of development, no toxicity or allergenicity studies have been conducted on the purified proteins expressed by the inserted genes. Bioinformatics searches for potential

allergens can be conducted as a predictive tool for identifying biologically relevant sequences or structural similarities to known allergens, although the results are not definitive and in general serve to indicate proteins requiring further attention (Goodman, 2008). They provide a good tool at early stages to indicate whether further testing of particular proteins should be considered. The amino acid sequences of the proteins expressed by the *AtBag4* and *TlBag4* genes were compared to sequences of known allergens using the AllergenOnline database, which contains data for over 2000 known allergens. These searches were made using parameters for the most predictive searches: overall FASTA alignment, with low E score values ($<1e^{-30}$) and/or identity matches over 50 % and additional searches using a sliding window of 80 amino acid searches looking for identities greater than 35%, as recommended for identifying allergenicity issues (Fiers et al., 2004). No relevant matches were found according to these parameters for the proteins encoded by the *AtBag4* or *TlBag4* genes to allergens listed in that database (information supplied by applicant).

42. As mentioned previously, the class of proteins expressed by the *Bag4* genes are highly conserved across a broad evolutionary distance, from single-cell yeasts to metazoans, including humans (Doukhanina et al., 2006; Kabbage et al., 2017). Thus homologues of the expressed proteins and proteins with very similar function occur naturally in a range of organisms including those routinely consumed by humans and other desirable animals. Based on this, it is likely that people and other beneficial organisms have a long history of exposure to the proteins expressed by the inserted genes. People handling GM chickpeas expressing the *AtBag4* or *TlBag4* genes in glasshouse trials have not reported adverse effects (supplied by applicant).

4.4 Characterisation of the GMOs

43. Although these lines are at an early stage of development, the applicant has provided preliminary information on expected phenotypes. Based on studies with *Bag* genes from rice (*Oryza sativa*) it is expected that expression of the *AtBag4* or *TlBag4* genes in GM chickpeas will result in increased drought and heat tolerance (information from applicant). In addition to their roles in tolerance of abiotic and biotic stress through putative induction of cell survival pathways and/or inhibition of PCD pathways, *Bag* genes may also regulate PCD functions involved in plant development (Doukhanina et al., 2006). Thus, it is possible that some plants expressing the inserted genes could show developmental changes. However, as these genes are expressing proteins with highly conserved functions across a wide range of organisms (Doukhanina et al., 2006; Rana et al., 2012; Kabbage et al., 2017) such effects are not expected in the GM chickpeas and no negative effects were noted in transformed chickpeas grown under glasshouse conditions (supplied by applicant). Comparison of morphological and physiological characteristics in GM rice plants expressing *AtBag4* indicated no significant differences between the GM and WT plants at seedling or reproductive stages (Hoang et al., 2015), indicating that expression of this gene in the GM plant is unlikely to result in changed plant development and morphology.

44. Glasshouse trials have shown that expression of the *AtBag4* and *TlBag4* gene in chickpea improved tolerance of drought, salinity and heat stress. Chickpeas expressing *AtBAG4* and *TlBag4* genes also had noticeably increased yield, related to increased seed number, when subjected to severe drought stress in the glasshouse, compared to non-GM chickpeas subjected to the same conditions (information supplied by the applicant).

Section 5 The receiving environment

45. The receiving environment forms part of the context in which the risks associated with dealings with the GMOs are assessed. Relevant information about the receiving environment includes abiotic and biotic interactions of the crop with the environment where the release would occur; agronomic practices for the crop; presence of plants that are sexually compatible with the GMO; and background presence of the gene(s) used in the genetic modification (OGTR, 2013).

46. Information relevant to the commercial cultivation and distribution of chickpeas in Australia, including key biotic and abiotic interactions in the chickpea-growing environment, is presented in the chickpea biology document (OGTR, 2019). Information relevant to the commercial cultivation and distribution of chickpeas in Australia is also available in a number of industry publications (Pulse Australia,

2015; GRDC, 2016; Pulse Australia, 2016; GRDC, 2017b, c, 2018; NSW DPI, 2018). Key factors are discussed below in Sections 5.1 to 5.3, with information summarised from these industry publications except where otherwise attributed.

5.1 Relevant biotic factors

47. Chickpeas are slow to emerge and grow, thus they are poor competitors with weeds, with yield losses of over 80% recorded in fields with uncontrolled weeds (Frenda et al., 2013; GRDC, 2017b). Weed control during seedling growth and into flowering are critical to preventing yield reduction. Broad-leafed weeds are particularly problematic in chickpea crops, as grass weeds can often be controlled with selective herbicides (GRDC, 2017b). Important weeds in eastern Australian chickpea crops include common sowthistle (*Sonchus oleraceus*), wild oats (*Avena spp*.) and turnip weed (*Rapistrum rugosum*) (Osten et al., 2007). Pre-emergent herbicides are the most common method of weed control for chickpeas in eastern Australia, followed by post-emergent herbicides (Osten et al., 2007). Inter-row cultivation and higher seeding rates are used less frequently. Sheep grazing may also be used to control weeds, e.g. volunteer peas, in chickpea crops, as chickpeas are less palatable than peas (GRDC, 2017b).

48. The major insect pest in Australian chickpea production is the native budworm, *Helicoverpa punctigera*, which reduces grain yield and quality when present during podding and grain-filling (GRDC, 2017b) . The chickpea plant's acidic secretions make it less attractive to insect pests than other pulses, however red-legged earth mite, lucerne flea, cutworms and aphids can cause damage during the emergence and seedling stages. Other insect pests such as locusts and grasshoppers can also cause damage to chickpeas but their effect is highly seasonal (Pulse Australia, 2015; GRDC, 2016; Pulse Australia, 2016; Agriculture Victoria, 2017; GRDC, 2017b, c). Insecticides are used to control insect pests when numbers exceed an economic threshold (GRDC, 2017b). Storage pests, such as weevils, are controlled with fumigation or controlled atmosphere treatment during postharvest storage (GRDC, 2017b).

49. The major nematode pests in Australian chickpeas are root lesion nematodes (*Pratylenchus spp*.), with the predominant species varying in different growing regions - *P. thornei* in the northern region of eastern Australia; *P. thornei* and *P. neglectus* in the southern region of eastern Australia; and *P. neglectus*, *P. quasitereoides*, *P. thornei* and *P. penetrans* in WA (GRDC, 2016, 2017b). No varieties are completely resistant, although there are varietal differences in resistance to *P. thornei* and *P. neglectus* and tolerant varieties can yield well when nematodes are present (NSW DPI, 2018). Chickpea is also susceptible to root-knot nematodes (*Meloidogyne spp*.), cyst-forming nematodes (*Heterodera spp*.) and reniform nematodes (*Rotylenchulus reniformis*) (Castillo et al., 2008). Avoiding nematode damage relies on farm hygiene, crop rotation and long fallows; no nematicides are registered for use in Australia (GRDC, 2016, 2017b, c).

50. A number of fungal and viral diseases are important in chickpea production. The fungal pathogen *Ascochyta rabiei* causes ascochyta blight, the major disease of chickpea in Australia and world-wide. Other important fungal diseases are botrytis grey mould (*Botrytis cinerea*), and sclerotinia stem and crown rot (*Sclerotinia* spp.). Phytophthora root rot is caused by a fungus-like oomycete (*Phytophthora medicaginis*). Root rot diseases caused by the *Fusarium* and *Rhizoctonia* fungi and oomycte *Pythium* spp. occur occasionally under wet conditions (GRDC, 2016, 2017b, c). The most important viral diseases of chickpea are those spread by aphids (Schwinghamer et al., 2009; NSW DPI, 2018). Luteoviruses are transmitted persistently by aphids, and include *Beet western yellows virus*, *Bean leafroll virus* and *Subterranean clover redleaf virus*. Non-persistently transmitted viruses include *Cucumber mosaic virus* and *Alfalfa mosaic virus*. Thrips and leafhoppers also transmit viruses. Virus control measures focus on reducing aphid infestation and removing sources of infection in alternate host plants (Pulse Australia, 2015; GRDC, 2016; Pulse Australia, 2016; Agriculture Victoria, 2017; GRDC, 2017b).

51. The major vertebrate pests of chickpeas in Australia are feral pigs, kangaroos, emus and brush-turkeys (in central Qld) (OGTR, 2019). Feral pigs and mice damage crops by digging up and eating germinating seeds and shoots (Coulston et al., 1993; Poole, 2011; GRDC, 2016) and overseas studies indicate that shallow-sown seed can also be predated by birds (van der Maesen, 1972).

52. Chickpea plants form symbioses with rhizobia that fix atmospheric nitrogen for use by the plant and subsequent crops and in Australia *Mesorhizobium ciceri* is used in commercial inoculants for chickpea

(GRDC, 2013). A number of factors influence persistence, which declines over time, so inoculation is recommended whenever leguminous crops are sown (GRDC, 2013). Arbuscular mycorrhizal fungi (AMF) colonisation of chickpea roots facilitates the extraction of phosphorus and zinc from the soil (GRDC, 2017b), in combination with acidic secretions from chickpea roots (Pulse Australia, 2016).

5.2 Relevant abiotic factors

53. It is proposed that the GMOs will be grown at the Walkamin Research Facility, Walkamin, Queensland, approximately 14 km north of Atherton and 70 km west of Cairns. This research station has been the site for research into a broad range of agriculture including tropical/subtropical food crops, trees, pasture and legumes, maize and sweetcorn development, tropical entomology, fruit fly, aquaculture and pigeon pea development. This property is over 600 km from the nearest commercial chickpea growing region.

54. Chickpea production is best suited to well-drained neutral to alkaline soils, from loams to self-mulching clays (GRDC, 2017b). Chickpea does not grow well in acid soils, sands, tight hard-setting clays, and soils that are saline, sodic or high in boron, or acid soils high in aluminium. Chickpeas are able to access atmospheric nitrogen through symbiotic relationships with rhizobia (GRDC, 2017b) and soil phosphorus and zinc through symbiosis with AMF (Pulse Australia, 2016).

55. Chickpea cultivars vary in sensitivity to temperature and day length (photoperiod) for flower initiation, allowing the species to be adapted to a range of growing environments (Berger et al., 2011). Although chickpeas are tolerant of cool conditions, frosts can be a problem in southern Australia, especially when they occur in the late vegetative and reproductive stages (GRDC, 2017b). Cool temperatures at flowering can lead to flower abortion (Toker et al., 2007; GRDC, 2017b) and pollen viability is reduced when plants are exposed to low temperature stress during pollen development (Clarke and Siddique, 2004), in some varieties low temperatures can also reduce fertilization.

56. Chickpea is sensitive to heat stress during flowering and podding and extended periods of high temperature during flowering leads to an increased rate of plant development, along with reduced biomass and yield (Kaushal et al., 2013). Heat stress can result in reduced pollen viability and germination. High temperatures during podding reduces biomass, number of seeds per plant and weight per seed (Wang et al., 2006).

57. Chickpeas are sensitive to both drought stress and the effects of waterlogging. Drought stress has greatest impact on chickpea yield when it occurs during flowering and podding (Khanna-Chopra and Sinha, 1987). Terminal drought in dryland crops reduces flower, pod and seed numbers; increases flower pod and seed abortion (Leport et al., 2006; Pang et al., 2017) and reduces the duration and rate of seed filling (Davies et al., 1999), thus reducing chickpea yield. Chickpeas are especially susceptible to waterlogging during flowering and podding (Cowie et al., 1996). Waterlogging can result in nutrient deficiency or plant death, with mortality rates increasing at later stages of development (GRDC, 2017b).

58. Chickpeas are susceptible to herbicide damage, particularly Group B sulfonyl urea herbicides (Pulse Australia, 2016), so consideration of spray drift and paddock history are important in growing chickpeas.

5.3 Relevant agricultural practices

59. Chickpeas are commercially cultivated in central and south eastern Queensland, in New South Wales, north western and western Victoria, southern areas of South Australia and south western areas of Western Australia. The proposed location for the trial is outside commercial production areas and experiences a higher rainfall than typical chickpea cultivation areas. However, this rainfall is concentrated over summer, with very low rainfall during the chickpea growing season (Bureau of Meteorology - Climate Data Online; accessed 5 December 2018).

60. The limits and controls of the proposed release are outlined in Section 2.1 and Section 2.2 of this Chapter. It is anticipated that the agronomic practices for the cultivation of the GM chickpea by the applicant will not differ significantly from industry best practices used in Australia. Some non-GM commercial lines of chickpea would be planted as controls for the trial.

61. The chickpeas would be planted 5 – 7 cm deep at a rate of 25 plants/m^2, with a row spacing of 0.5 m, into soil with good moisture content. Chickpeas would be planted in June or July and harvested in November or December, dependent on seasonal conditions and maintained using commercial practices for chickpea production. Although the crop would be managed as a dryland crop, channel or drip irrigation may be used if required. Applications of any agricultural chemicals would be in accordance with APVMA requirements and may include herbicides such as glyphosate or diquat, insecticides such as carbamates or pyrethroids and fungicides such as carbendazim, chlorothalonil and mancozeb when appropriate, as recommended in industry (GRDC, 2016). Harvesting of the seeds would be by small plot harvester. Following harvest the land would be left as fallow until planted with chickpea for the trial in the following season.

5.4 Presence of related plants in the receiving environment

62. Chickpeas are grown commercially in Australia over a wide range of cropping areas, however, the trial site is over 600 km from the nearest commercial chickpea growing area – Region 1: Low rainfall tropical (Pulse Australia, 2016). The applicant has confirmed that no other chickpeas trials would be planted at the research station while GM chickpeas are being grown. No sexually compatible species are present in Australia.

5.5 Presence of similar genes and encoded proteins in the environment

63. The introduced genes and regulatory sequences were isolated from commonly occurring organisms that are already widespread in the environment (see Table 1, section 4.1).

64. Programmed cell death (PCD) is an integral part of plant and animal tissue development (see Section 4.2 of this chapter). Multicellular organisms in which apoptosis is a normal function already contain anti-apoptotic genes. Therefore, it is expected that humans, animals and microorganisms routinely encounter the introduced genes for inhibition of apoptosis, homologues of these genes and their expressed proteins (or proteins with a similar function), through contact with plants and food derived from plants.

65. The regulatory sequences that control expression of the genes inserted in the GM chickpeas are derived from microorganisms that are common in the environment (Cauliflower mosaic virus (CaMV), Subterranean clover stunt virus (SCSV) and *A. tumefaciens*), as mentioned in Section 4.1. Humans and animals are routinely exposed to these in the environment.

66. The GM chickpea plants also contain the *nptII* selectable marker gene derived from *E. coli* a common bacterium that is widespread in human and animal digestive systems and/or in the environment. Both humans and animals are routinely exposed to these genes and their encoded proteins. More information on marker genes is available in the document Marker Genes in GM Plants.

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

67. There have been no approvals for field trials or commercial release of GM chickpea in Australia.

6.2 International approvals

68. One GM chickpea trial is listed in the United States in 2006 - 2007, for an insect resistant chickpea, but no commercial release is recorded (USDA APHIS Biotechnology Permits, accessed 24 January 2019) and some studies have been conducted under controlled conditions to examine the possibilities for GM chickpea (OGTR, 2019 and references therein). However, no general releases are recorded (European Union GM Register; International Service for the Acquisition of Agri-Biotech Applications (ISAAA) GM Approval database; all accessed 24 January 2019).

69. None of the lines in the current application have been approved for release in any other country.

Chapter 2 Risk assessment

Section 1 Introduction

70. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 2). Risks are identified within the established risk assessment context (Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

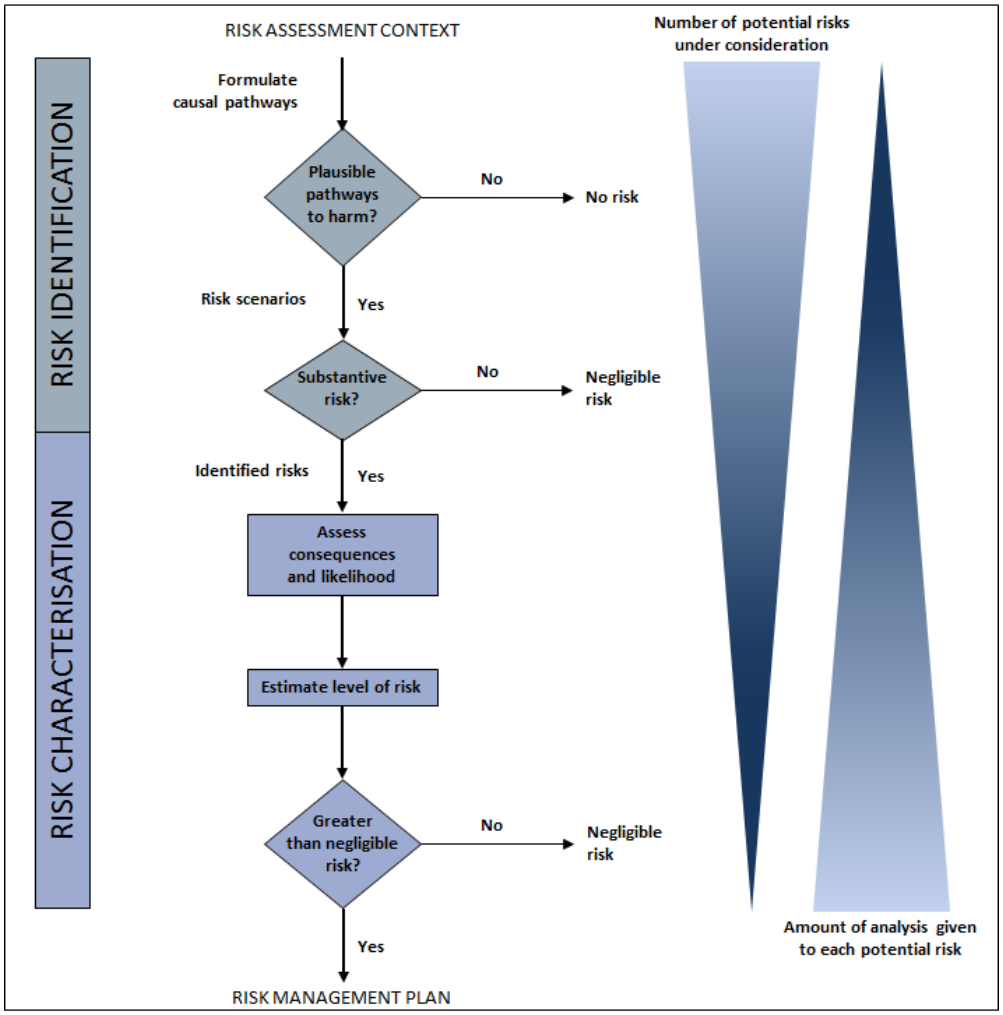

Figure 2. The risk assessment process

71. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, reported international experience and consultation (OGTR, 2013). A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants, as this approach addresses the full range of potential adverse outcomes associated with plants. In particular, novel traits that may increase the potential of the GMO to spread and persist in the environment or increase the level of potential harm compared with the parental plant(s) are used to postulate risk scenarios (Keese et al., 2014). Risk scenarios examined in RARMPs prepared for licence applications for the same or similar GMOs, are also considered.

72. Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating plausible causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are risk scenarios. These risk scenarios are screened to identify those that are considered to have a reasonable chance of causing harm in the short or long term. Pathways that do not lead to harm, or

those that could not plausibly occur, do not advance in the risk assessment process (Figure 2) i.e. the risk is considered to be no greater than negligible.

73. Risks identified as being potentially greater than negligible are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). Risk evaluation then combines the Consequence and Likelihood assessments to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 **Risk Identification**

74. Postulated risk scenarios are comprised of three components (Figure 3):

i. the source of potential harm (risk source)

ii. a plausible causal linkage to potential harm (causal pathway)

iii. potential harm to people or the environment.

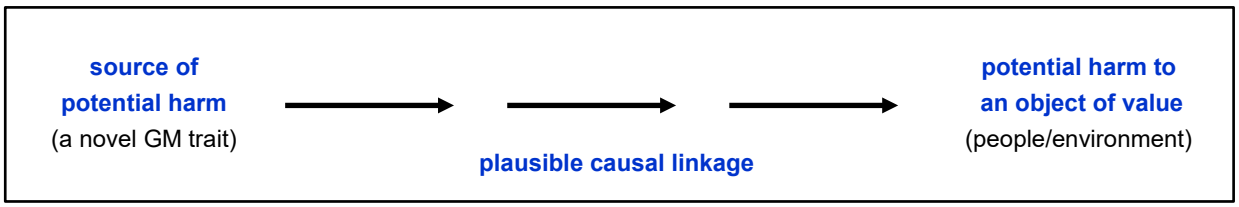

Figure 3: Risk scenario

75. When postulating relevant risk scenarios, the risk context is taken into account, including the following factors detailed in Chapter 1:

- the proposed dealings
- the proposed limits including the extent and scale of the proposed dealings
- the proposed controls to limit the spread and persistence of the GMO and
- the characteristics of the parent organism(s).

2.1 Risk source

76. The sources of potential harms can be intended novel GM traits associated with one or more introduced genetic elements, or unintended effects/traits arising from the use of gene technology.

77. As discussed in Chapter 1, the GM chickpea lines have been modified by the introduction of the *AtBAG4* and *TlBAG4* genes derived from *A. thaliana* and *T. loliiformis* respectively. The intended effects of insertion of these genes is to increase tolerance to drought and heat, although the genes may also be involved in tolerance to other abiotic stresses. These introduced genes are considered further as potential sources of risk.

78. The GM chickpea also contains the marker gene *nptII* from *E. coli* that confers antibiotic resistance and was used as a selectable marker gene. This gene and its product have been extensively characterised and assessed as posing negligible risk to human or animal health or to the environment by the Regulator, as well as by other regulatory agencies in Australia and overseas. Further information about this gene can be found in the document *Marker genes in GM plants* available from the Risk Assessment References page on the OGTR website. As the gene has not been found to pose a substantive risk to either people or the environment, its potential effects will not be further considered for this application.

79. The introduced genes are controlled by introduced regulatory sequences. These were derived from cauliflower mosaic virus, subterranean clover stunt virus and *A. tumefaciens*. Regulatory sequences are naturally present in all plants and the introduced sequences are expected to operate in similar ways to

endogenous sequences. These sequences are DNA that is not expressed as a protein, so exposure is to the DNA only and dietary DNA has no toxicity (Society of Toxicology, 2003). Hence, potential harms from the regulatory sequences will not be further assessed for this application.

80. The genetic modifications have the potential to cause unintended effects in several ways. These include insertional effects such as interruptions, deletions, duplications or rearrangements of the genome, which can lead to altered expression of endogenous genes. There could also be increased metabolic burden due to expression of the introduced proteins, novel traits arising out of interactions with non-target proteins and secondary effects arising from altered substrate or product levels in biochemical pathways. However, these types of effects also occur spontaneously and in plants generated by conventional breeding (Ladics et al., 2015; Schnell et al., 2015). Accepted conventional breeding techniques such as hybridisation, mutagenesis and somaclonal variation can have a much larger impact on the plant genome than genetic engineering (Schnell et al., 2015; Anderson et al., 2016). Plants generated by conventional breeding have a long history of safe use, with few documented cases where conventional breeding has resulted in an unacceptable level of a metabolite in a crop (Berkley et al., 1986; Seligman et al., 1987). There are no documented cases where conventional breeding has resulted in the production of a novel toxin or allergen in a crop (Steiner et al., 2013). Current practices identify and remove harmful non-GM plants to protect domesticated animals and people (Steiner et al., 2013). Therefore, the potential for the processes of genetic modification to result in unintended effects will not be considered further.

2.2 Causal pathway

81. The following factors are taken into account when postulating plausible causal pathways to potential harm:

- 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) on the properties of the organism
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the environment at the site(s) of release
- agronomic management practices for the GMOs
- spread and persistence of the GM plants (e.g. reproductive characteristics, dispersal pathways and establishment potential)
- tolerance to abiotic conditions (e.g. climate, soil and rainfall patterns)
- tolerance to biotic stressors (e.g. pest, pathogens and weeds)
- tolerance to cultivation management practices
- gene transfer to sexually compatible organism
- gene transfer by horizontal gene transfer
- unauthorised activities.

82. Although all of these factors are taken into account, some are not included in the risk scenarios below as they may have been considered in previous RARMPs and a plausible pathway to harm could not be identified.

83. The potential for horizontal gene transfer (HGT) from GMOs to species that are not sexually compatible, and any possible adverse outcomes, have been reviewed in the literature (Keese, 2008) and assessed in many previous RARMPs. HGT was most recently considered in the RARMP for DIR 108. Although the DIR 108 RARMP is for GM canola, the HGT considerations are the same for the current RARMP: plant HGT events rarely occur and the wild-type gene sequences or homologues are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, no substantive risk was identified in previous assessments and HGT will not be further considered for this application.

84. The potential for unauthorised activities to lead to an adverse outcome has been considered in many previous RARMPs, most recently in the RARMP for DIR 117. In previous assessments of unauthorised activities, no substantive risk was identified. The Act provides substantial penalties for unauthorised dealings with GMOs or noncompliance with licence conditions, and also requires the Regulator to have

regard to the suitability of an applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, risks from unauthorised activities will not be considered further.

2.3 Potential harm

85. Potential harms from GM plants are based on those used to assess risk from weeds (Virtue, 2008; Keese et al., 2014) including:

- harm to the health of people or desirable organisms, including toxicity/allergenicity
- reduced biodiversity through harm to other organisms or ecosystems
- reduced establishment or yield of desirable plants
- reduced products or services from the land use
- restricted movement of people, animals, vehicles, machinery and/or water
- reduced quality of the biotic environment (e.g. providing food or shelter for pests or pathogens) or abiotic environment (e.g. negative effects on fire regimes, nutrient levels, soil salinity, soil stability or soil water table).

86. Judgements of what is considered harm depend on the management objectives of the land where the GM plant may be present. A plant species may have different weed risk potential in different land uses such as dryland cropping or nature conservation.

2.4 Postulated risk scenarios

87. Three risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 2 and examined in detail in Sections 2.4.1 – 2.4.3.

88. In the context of the activities proposed by the applicant and considering both the short and long term, none of the three risk scenarios gave rise to any substantive risks.

Table 2: Summary of risk scenarios from the proposed dealings with the GM chickpea

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced genes conferring increased drought tolerance	Growing GM chickpea at the field trial sites ⬇ Expression of the introduced genes in GM plants ⬇ Exposure of humans or other desirable organisms by ingestion of, or contact with, the plant material	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms	No	• No known toxicity or allergenicity for the inserted genes and their expressed proteins • Encoded proteins and similar proteins occur naturally in the environment and are not known to be toxic or allergenic to people or other desirable organisms • No reason to expect that novel proteins would be expressed in GM hybrids nor that the expressed proteins would behave differently in a hybrid background • The small size and short duration of the proposed trial would minimise exposure of people and other desirable organisms to the GM plant material • No food or feed to be produced from this trial

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
2	Introduced genes conferring increased drought tolerance	Growing GM chickpea at the field trial sites ⬇ Dispersal of GM seed outside the trial limits ⬇ GM seed germinates ⬇ Establishment of GM chickpea plants in nature reserves, roadside areas or intensive use areas	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms OR Reduced establishment and yield of desirable plants	No	• Proposed limits and controls minimise the likelihood of seed dispersal outside the trial site • There is no expectation the introduced gene constructs confer other characteristics to enhance the spread and persistence of the GM chickpeas • Chickpeas are unlikely to be dispersed by animals • Chickpeas have limited ability to survive outside agricultural settings • The GM chickpeas can be controlled using conventional methods • Scenario 1 did not identify an increased risk of allergenicity or toxicity in the GM chickpeas
3	Introduced genes conferring increased drought tolerance	Growing GM chickpeas at the field trial sites ⬇ Fertilisation of sexually compatible plants outside the trial site by pollen from GM chickpea plants ⬇ Germination of GM hybrid seed ⬇ Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms OR Reduced establishment and yield of desirable plants	No	• Proposed limits and controls minimise the likelihood of pollen dispersal outside the trial site • There is no expectation the introduced gene constructs confer other characteristics to enhance the spread and persistence of the GM chickpeas • There are no sexually compatible species with which chickpeas can hybridise • There is no indication that hybrid plants would have increased ability to survive outside agricultural settings • Risk scenarios 1 and 2 did not identify toxicity, allergenicity or weediness of the GMOs as substantive risks.

2.4.1 Risk scenario 1

Risk Source	Introduced genes conferring increased drought tolerance
Causal Pathway	⬇ GM chickpeas are planted at the field trial site ⬇ Expression of the introduced genes in GM plants ⬇ Exposure of humans or other desirable organisms by ingestion of, or contact with, the plant material ⬇
Potential Harm	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms

Risk source

89. The source of potential harm for this postulated risk scenario is the introduced genes for drought tolerance in GM chickpea lines.

Causal pathway

90. The GM chickpea plants are planted at the field trial site and the genes for abiotic stress tolerance are expressed. The proteins encoded by the inserted genes are under the control of a constitutive promoter, so they may be expressed in all plant tissues.

91. People may be exposed to GM plant material and the expressed proteins, either by direct contact with the plant material or through inhalation of pollen. This is most likely at the trial site, but could also occur during transport and handling of GM plant material. Other organisms such as livestock, rodents, marsupials, birds or invertebrates may be exposed at the trial site through contact with, or ingestion of GM plant material. Chickpea pollen matures and is released from the anthers while the flower is at the half-open stage, thus chickpeas are almost entirely self pollinated and pollen release is limited. This limits the exposure of people or other desirable organisms to chickpea pollen.

92. The trial is proposed for a maximum of six growing seasons during the period from July 2019 until December 2024. The potential for exposure is limited to a short period when GMOs are present at the trial sites during these growing seasons (June/July until November/December). The proposed planting area is a maximum of 3 ha per season at a single site located on land owned and controlled by QDAF that would only be accessed by authorised people. Transport and storage of the GM plant material would be conducted according to the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs, thus limiting exposure of people during transport and storage of the GMOs. No material from this trial would be used for human food or animal feed. These proposed limits and controls would minimise the exposure of people or animals to the GM plants and their products.

Potential harm

93. 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). Allergenicity is the potential of a substance 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).

94. Potentially, people exposed to the proteins expressed by the introduced genes may show increased toxic reactions or increased allergenicity. Similarly, exposure to the proteins expressed by the introduced genes may lead to increased toxicity to other desirable organisms. From consideration of the causal pathway, including the proposed limits and controls, human exposure would be limited to staff involved in handling the GM chickpea plants during the course of the field trial.

95. Although no toxicity or allergenicity studies have been performed on the GM plant material or the expressed proteins, the applicant has supplied information from bioinformatic searches of the amino acid sequences for the expressed proteins. These searches yielded no matches with known allergens. In addition, the GM chickpeas have been grown in the glasshouse, with no reports of adverse effects from people dealing with the plants.

96. As discussed in Chapter 1 (Section 3) and in the biology document (OGTR, 2019) chickpeas are primarily a food crop and although non-GM chickpeas produce some toxins and anti-nutritional factors, these are generally reduced during the preparation of chickpeas for food. Likewise, there are records of allergies to chickpeas, usually in conjunction with high consumption rates and allergies to other legumes. However, there is no reasonable expectation that the genes expressed in the GM chickpeas would affect the pathways producing known toxins or allergens in chickpea or lead to the production of novel toxins or allergens.

97. The inserted genes are involved in PCD, which is an integral part of plant and animal development, as well as being involved in responses to environmental stresses. Thus, such anti-apoptotic genes are present in a range of organisms in the environment. As such, humans and other beneficial organisms routinely encounter the introduced genes or homologues of these genes and their products through contact with plants or animals and food derived from them, as well as potentially expressing homologues of these genes themselves. Thus, it is highly unlikely that there would be any effect greater than that seen from non-GM chickpeas on any humans or other desirable organisms, including insects, exposed to the crop.

98. Additionally, it is proposed that large animals would be excluded from the trial site whilst GM chickpeas are growing and that the chickpeas from this trial will not be used for human food or animal feed, thus further limiting the exposure of humans and other desirable organisms to the GM chickpeas.

Conclusion

99. Risk scenario 1 is not identified as a substantive risk due to limited exposure and the lack of toxicity or allergenicity of the introduced genes and their encoded proteins to humans and lack of toxicity to other organisms. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk Source	Introduced genes conferring increased drought tolerance
Causal Pathway	Growing GM chickpea at the field trial sites ⬇ Dispersal of GM seed outside the trial limits ⬇ GM seed germinates ⬇ Establishment of GM chickpea plants in nature reserves, roadside areas or intensive use areas ⬇
Potential Harm	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms OR Reduced establishment and yield of desirable plants

Risk source

100. The source of potential harm for this postulated risk scenario is the introduced genes for drought tolerance in GM chickpea lines.

Causal pathway

101. If GM chickpea seed was dispersed outside the trial sites, or persisted at the trial sites after completion of the trial, this seed could germinate and give rise to plants expressing the introduced genes. These plants could spread and persist in the environment and establish populations of GM chickpeas, expressing genes for increased drought tolerance. This could increase the likelihood of exposure of people or other desirable organisms to the proteins expressed in the GM plants.

102. Morphological and physiological characteristics of chickpeas would limit the likelihood of spread and persistence in the environment. The pods of the chickpea ancestor, *C. reticulatum*, do not dehisce and shatter once dry, thus, unlike many crop species, domestication did not require selection to alter this character for commercial chickpeas. Commercial chickpeas have non-dehiscent pods (van der Maesen, 1972) and show less tendency to shed pods or shatter seeds than this ancestral species (Ladizinsky, 1979). However, weathering and crop morphology can result in harvest losses of 5–30% (Loss et al., 1998; GRDC, 2017b), thus seed could remain at the trial site.

103. As outlined in Chapter 1, section 3, chickpeas do not display weedy characteristics. Although commercial chickpea seeds may survive from one season to the next under natural conditions (Auckland and van der Maesen, 1980), there is no evidence for dormancy in chickpeas (MoEF&CC, 2016; OGTR, 2019). Under field conditions chickpea seed can survive for several years, if seed is buried and the soil remains dry, or if seed is left on the soil surface and does not imbibe sufficient moisture to germinate (OGTR, 2019). In paddocks that have not received sufficient moisture for germination, i.e. during drought conditions, chickpeas can remain in the seed bank and germinate 2 - 3 years after the previous crop. However, high moisture conditions, including rainfall after harvest, high temperatures and physical damage reduce seed viability (Loss et al., 1998). The trial site is in an area that receives high rainfall after harvest - on average about 790 mm from November to March - and high temperatures (Bureau of Meteorology - Climate Data Online; accessed 5 December 2018) [4], thus seeds would germinate and be unlikely to remain viable at the

[4] The QDAF website lists rainfall at Walkamin research Facility as 760 mm per year. However data from Bureau of Meteorology show mean rainfall at Bureau station 031108 (Walkamin Research Station) as 1017.3 mm (median 963.1 mm). However, using either information source, approximately 70 % rainfall falls between December and March.

trial site. Additionally proposed controls would require inspection and cleaning of any areas used to grow the GMOs in this trial and destruction of any viable plant material.

104. If any seeds were to survive and germinate, chickpeas are poor competitors with weeds and do not establish well in competition with other plants. Therefore in natural environments, where other plants are present it is unlikely that chickpea seedlings would survive and establish. Volunteers do occasionally occur in areas such as roadsides where seed is spread, but most often do not survive to produce viable plants. They are most commonly observed in areas where chickpea crops have been grown, as volunteers in subsequent crops, where they would be controlled by conventional weed control methods (OGTR, 2019).

105. The most likely means of dispersal of chickpea seeds outside the trial site are through the activities of people or animals or through extreme weather events.

Dispersal through human activity

106. Although human activity is a likely mechanism for seed dispersal from chickpea crops, the applicant has proposed limits and controls to prevent the spread of GM chickpea seed from the trial site. Access to the site is restricted to authorised, trained staff. The applicant has proposed harvesting using dedicated small plot harvesters and all equipment used at the trial site would be cleaned before being used for any other purpose. All GM plant material would be transported in accordance with the Regulator's Transport, Storage and Disposal of GMOs guidelines, which would minimise the opportunity for dispersal of GM material or for contact with any GM plant material during transport from the trial site to QUT facilities for analysis.

Dispersal by animals

107. A number of non-flying animals are regarded as pests of chickpeas and will eat seeds and young shoots. Feral pigs seek out germinating chickpea seeds and cause major damage to crops (GRDC, 2016) and, when present in plague proportions, mice will eat chickpea seed and emerging shoots (Coulston et al., 1993; Poole, 2011). Other animals such as emus, brush turkeys (in central Queensland) and kangaroos will also eat chickpea seed (OGTR, 2019). It is not known whether viable seeds survive passage through the gut of these animals. However, the large seed size (Desi chickpea seeds are 80 – 350 mg (Knights and Hobson, 2016), compared to wheat seeds of approximately 40 mg) means that seeds are likely to be damaged by chewing and are likely to be rendered non-viable by imbibing liquid during digestion. Whether mice or other rodents actively move chickpea seed from crops is not known, although given the large seed size this would appear unlikely and it is also logical to expect that seed would be damaged during movement by rodents. Chickpea seed may be moved in pig faeces, but it is not clear whether such seed remains viable. It is also possible that emus may move seed in their beaks, but seeds in faeces have not been observed (OGTR, 2019). Other native animals may move through chickpea crops, however little is known about whether they actively feed on chickpea seeds and if so, whether they transport viable seeds to other areas (OGTR, 2019). Chickpea crops also become less palatable as they mature (Mayfield et al., 2008) and thus less attractive for animal feeding, particularly if other sources of feed are available. Overall, there is no evidence that wild animals play a role in the dispersal of chickpeas.

108. While whole chickpea (non-GM) may be used as stockfeed, the viability of chickpea seed after passing through the digestive tract of different animals is poorly understood. A study on the passage of ten different legume seeds, not including chickpea, through bovine rumen found that ingested soft (germinable) seed was unlikely to be recovered from faeces in a viable state (Gardener et al., 1993). Soft seeds imbibe water in the digestive tract and become vulnerable to the digestive process. Large seeds such as chickpea are also more prone to damage from chewing and rumination than small seeds (OGTR, 2019). Additionally, chickpea seeds lack physical characteristics that generally enable transport in fur or feathers, or in mud on the legs or feet of animals or birds, so this type of seed movement is unlikely.

109. While there are reports from other countries of birds feeding on chickpea crops (van der Maesen, 1972), there is limited information on predation by Australian bird species, such as cockatoos and galahs and their ability to disperse viable chickpea seed is unknown (OGTR, 2019). However, there is no evidence that flying animals play a role in the dispersal of chickpeas in Australia.

110. While there are a number of insect pests that damage chickpea crops by feeding on plant tissues, no reports were found of insects removing or spreading chickpea seeds from cropping areas.

111. The proposed trial sites are small and the period during sowing and immediately after harvest, when animals could consume or spread viable seeds, is short. The applicant proposes to maintain the monitoring zone surrounding chickpea planting areas as fallow and also proposes the use of mouse baits as required during the field trial. These measures would assist in rodent control at the trial site. They are proposing to fence the trial site, with fences positioned around the planting area and associated monitoring and isolation zones, to prevent access by feral pigs. Such a fence would also limit access by livestock and some other large animals. However, the weed risk assessment for chickpeas concluded that spread in this way is 'unlikely to occasional' and that there is no evidence that wild animals play a role in chickpea dispersal. It is considered that spread by livestock would only be occasional as chickpeas are likely to be damaged during digestion and therefore viable seed would rarely be spread (OGTR, 2019). In addition, no use as animal feed is proposed in this trial.

Dispersal in extreme weather

112. Extreme weather events have the potential to spread plant material outside a trial, with the most likely means of spread through wind or water. While plant material such as leaves, stalks or indeed whole plants may be moved short distances by extreme winds, it is not clear that this could move plant material outside the trial site. It is unlikely that chickpea seed would be spread by wind as seeds are heavy and they lack specific structures associated with wind transport. Dispersal by water is possible, but is unlikely as chickpea seeds are heavy and not adapted for water dispersal. It is also proposed that trial sites will be at least 100 m from any natural watercourse or manmade watercourses that flow into natural watercourses and in areas that are not prone to flooding.

Potential Harm

113. If GM plants were able to establish outside the trial site they could potentially cause increased toxicity or allergenicity to humans or increased toxicity to other desirable organisms through increased exposure. However, as discussed in Chapter 1 (section 4.3) and in Risk Scenario 1, there is no reasonable expectation that the GM chickpeas and their products, alone or in combination through hybridisation, would be any more toxic or allergenic than non-GM chickpeas.

114. Establishment of GM chickpeas outside the trial site could potentially reduce the establishment and/or yield of desirable plants by a number of means. This could occur through reduced establishment or yield of desirable agricultural crops; reduced establishment of desirable native vegetation; reduced utility of roadsides, drains, channels and other intensive use areas; or by providing a reservoir for pathogens or pests.

115. As discussed in Chapter 1 (Section 3) and in *The biology of* Cicer arietinum *L. (chickpea)* (OGTR, 2019), non-GM chickpeas are not regarded as weeds, either in Australia or internationally (Groves et al., 2003; Randall, 2017) and weedy populations are rarely found outside cultivated areas (OGTR, 2019).

116. The GM chickpeas proposed for this trial express genes that are expected to increase the chickpeas' tolerance to drought. In particular the applicants expect increased drought tolerance to be achieved by increased tolerance to heat stress or to water stress or both. In addition, tolerance to other abiotic stress such as UV, cold or oxidative stress may also be increased by the expression of these genes (Doukhanina et al., 2006; Kabbage and Dickman, 2008; Hoang et al., 2015; Kabbage et al., 2017). Thus under drought conditions – and potentially other environmental stresses – the GM chickpea plants may survive and reproduce more successfully than non-GM chickpeas. Under drought conditions in the glasshouse GM chickpeas had significantly higher yields than comparable non-GM chickpea lines (information supplied by the applicant). The expressed genes in the GM lines may result in improved resistance to necrotrophic pathogens, however this has not been tested (information supplied by the applicant).

117. However, in order to increase weediness, these characteristics would need to be coupled with other mechanisms that increase spread and persistence in the environment, through changes in dispersal,

establishment and survival. These characteristics would not reasonably be expected to change as a result of the introduced genes, either in individual lines or in a hybrid background.

118. Additionally, chickpea establishment and survival is limited by a number of other factors, such as disease, poor ability to compete with weeds, sensitivity to acidic or alkaline soils, mineral toxicities and sensitivity to certain classes of herbicides. Although the applicant mentions that the GM chickpeas may have improved resistance to some pathogens, this is untested as yet and there is no reasonable expectation that expression of the inserted genes would change the GM chickpeas' ability to establish, survive and persist in the presence of the other limiting factors. Also, optimal chickpea yields are generally achieved only with human intervention such as weed control and inoculation of seeds with *Rhizobium* - which are neither present nor persistent in Australian soils (GRDC, 2017a) - to assist with nodulation and nitrogen fixation, so growth and yields of plants growing outside cultivation are likely to be reduced.

119. None of the introduced traits are likely to change the susceptibility of the GM chickpea lines to conventional controls. Thus, if required, the GM chickpea plants proposed in this trial could be controlled by standard weed control measures, such as cultivation or the use of herbicides.

120. The limits and controls outlined in Risk Scenario 1 reduce the potential amount of seed available for dispersal outside the trial site, as well as the opportunities for spreading seeds. Additionally, Risk Scenario 1 did not identify toxicity or allergenicity of any of the individual genes or combinations of the introduced genes in a GM hybrid background, as a substantive risk. Thus even if spread of seed occurred and increased the likelihood of exposure to the GMOs, there is no reasonable expectation of increased toxicity or allergenicity to people or toxicity to other beneficial organisms.

Conclusion

121. Risk scenario 2 is not identified as a substantive risk due to the lack of toxicity or allergenicity of the introduced genes and their encoded proteins; the proposed limits and controls designed to restrict dispersal; the extremely limited ability of the GM chickpea to spread and persist outside the trial site and their susceptibility to standard weed control measures. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk scenario 3

Risk Source	Introduced genes conferring increased drought tolerance
Causal Pathway	Growing GM chickpeas at the field trial sites ⬇ Fertilisation of sexually compatible plants inside or outside the trial site by pollen from GM chickpea plants ⬇ Germination of GM hybrid seed ⬇ Spread and persistence of GM hybrid plants in nature reserves, roadside areas or intensive use areas ⬇
Potential Harm	Increased toxicity or allergenicity for humans or increased toxicity to other desirable organisms OR Reduced establishment and yield of desirable plants

Risk source

122. The source of potential harm for this postulated risk scenario is the introduced genes for drought tolerance in GM chickpea lines.

Causal pathway

123. Pollen from GM chickpea lines could fertilise sexually compatible plants either inside or outside the trial sites. Hybrid plants carrying the inserted genes could form the basis for spread and dispersal of these genes in other varieties of chickpea, or other sexually compatible plant species. People and other desirable

organisms could then be exposed to the proteins expressed by the introduced genes through ingestion, contact with plant material or inhalation of pollen from hybrid plants.

124. It should be noted that vertical gene flow per se is not considered an adverse outcome, but may be a link in a chain of events that may lead to an adverse outcome. Baseline information on vertical gene transfer associated with non-GM chickpea plants can be found in the chickpea biology document and a summary is provided in Chapter 1, Section 3 of this RARMP.

125. Chickpeas are largely self-pollinating and outcrossing rates within close plantings are on average, less than two percent. There is also no evidence that insect or animal pollination increases seed production in chickpeas (Klein et al., 2007), despite observations of insects visiting open chickpea flowers (van der Maesen, 1972; Tayyar et al., 1996). The proposed trial consists of up to 60 lines of GM chickpeas, each containing one of two abiotic stress tolerance genes, with non-GM chickpeas grown within the trial as comparators. It is possible, that GM chickpea lines could cross-pollinate or that they could pollinate the non-GM chickpeas grown as part of the trial.

126. If pollen flow between chickpea lines containing different stress tolerance genes occurred, hybrid lines containing two abiotic stress tolerance genes could result. However, this is highly unlikely given the low outcrossing rates reported for chickpeas (see Chapter 1, Section 3). In addition, there are requirements for any volunteers at the trial site to be destroyed before flowering, so in the very rare case that a hybrid plant occurred, it would not be allowed to remain and set seed.

127. As discussed in Chapter 1 (Section 3), the primary gene pool for chickpea consists of two species, *C. reticulatum* and *C. echinospermum*, neither of which is listed as weedy (Randall, 2017) and neither species is cultivated. These species are not present in Australia and given that they are not crop plants and Australia has strict biosecurity regulations, it is unlikely that either species would be brought to Australia, thus there is no reasonable risk of outcrossing to related species.

128. The proposed limits and controls for this trial would minimise the likelihood of pollen flow from the trial to non-GM chickpeas outside the trial site. Under the proposed conditions, no chickpeas may be present within at least 8 m of a planting area while GM chickpea lines are being cultivated and any chickpeas must be controlled within this distance during flowering. This would greatly reduce the already low potential for pollen flow from the trial to chickpeas planted outside the trial sites. Additionally, the applicant has stated that no other chickpeas would be grown at the research station while GM chickpea trials are in progress. The research station is located over 600 km from the nearest commercial chickpea cultivation areas.

129. The applicant proposes postharvest monitoring of the sites for any volunteer GM chickpea to prevent production of plants that could hybridise with other chickpeas through pollen flow.

Potential Harm

130. If pollen from GM chickpea lines was dispersed, resulting hybrids could spread and persist in the environment, leading to increased exposure and potentially increased toxicity or allergenicity to humans or increased toxicity to other beneficial organisms. Hybrids expressing the introduced genes could also reduce the establishment and yield of desired plants and subsequently reduce biodiversity.

131. If hybrids between two GM chickpea lines were to occur they could contain two genes for increased abiotic stress tolerance. However, they would not be expected to show different traits from either of the GM lines from which they were derived, nor expected to produce any novel products or show any difference in toxicity or allergenicity from either GM parent. Hybrids between GM chickpeas and non-GM chickpeas would result in progeny with the same gene for increased abiotic stress tolerance as the GM parent. However, there is no reason to believe that hybrid plants would possess a level of toxicity or allergenicity greater than that of either parent. Nor is it likely that such hybrids would possess a level of weediness greater than that of either parent.

132. In the rare event of vertical transfer from the GM chickpea lines to non-GM chickpea lines, it is expected that the introduced genes would confer the same properties in the hybrid as the GM parent.

Thus, as discussed in Risk scenarios 1 and 2, the introduced gene products, are not expected to be toxic to humans or other organisms, nor are they likely to make the chickpea lines more weedy. These characteristics are not expected to differ in a hybrid background.

133. The location of the trial site and the proposed isolation distance, together with the lack of any related species in Australia, greatly restrict the possibility of pollen flow and subsequent vertical gene transfer of the genes from the GM lines to any plants outside the trial planting area.

Conclusion

134. Risk scenario 3 is not identified as a substantive risk due to the limited possibility of pollen flow for chickpeas. In addition, Risk scenarios 1 and 2 did not identify toxicity, allergenicity or weediness of the GMOs as substantive risks. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 **Uncertainty**

135. Uncertainty is an intrinsic part of risk and is present in all aspects of risk analysis. This is discussed in detail in the Regulator's Risk Analysis Framework document.

136. Uncertainty is addressed by approaches such as balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios involving uncertainty to lead to harm. If there is residual uncertainty that is important to estimating the level of risk, the Regulator will take this uncertainty into account in making decisions.

137. As field trials of GMOs are designed to gather data, there are generally data gaps when assessing the risks of a field trial application. However, field trial applications are required to be limited and controlled. Even if there is uncertainty about the characteristics of a GMO, limits and controls restrict exposure to the GMO, and thus decrease the likelihood of harm.

138. For DIR 166, uncertainty is noted particularly in relation to:

- potential increased toxicity of GM chickpea to people or animals
- potential increased allergenicity to people
- potential for the genetic modification to have any improved abiotic or biotic stress tolerance or changes to other cell death pathways that could lead to increased spread and persistence of the GMOs

139. Additional data, including information to address these uncertainties, may be required to assess possible future applications with reduced limits and controls, such as a larger scale trial or the commercial release of these GMOs.

140. Chapter 3, Section 4, discusses information that may be required for future release.

Section 4 **Risk evaluation**

141. Risk is evaluated against the objective of protecting the health and safety of people and the environment to determine the level of concern and, subsequently, the need for controls to mitigate or reduce risk. Risk evaluation may also aid consideration of whether the proposed dealings should be authorised, need further assessment, or require collection of additional information.

142. Factors used to determine which risks need treatment may include:

- level of risk
- uncertainty associated with risk characterisation
- interactions between substantive risks.

143. Three risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the control measures proposed by the applicant, and

considering both the short and long term, none of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 2 and include:

- the introduced genes and their expressed proteins are unlikely to be toxic or allergenic
- no GM plant material would enter human food or animal feed
- limits on the size and duration of the proposed release
- suitability of proposed controls to restrict the spread and persistence of the GM chickpeas and its genetic material
- GM chickpea has limited ability to survive outside cultivation
- GM chickpea volunteers could be controlled by conventional weed control measures

144. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM chickpea plants into the environment are considered negligible. The *Risk Analysis Framework* (OGTR, 2013), which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.[5]

[5] As none of the proposed dealings are considered to pose a significant risk to people or the environment, Section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator has allowed 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities, and the public.

Chapter 3 Risk management plan

Section 1 Background

145. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan addresses risks evaluated as requiring treatment and considers limits and controls proposed by the applicant, as well as general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through licence conditions.

146. 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.

147. All licences are subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: Section 64 requires the licence holder to provide access to premises to OGTR inspectors 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 must also be reported to the Regulator.

148. The licence is also 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 to manage risk to people or the environment. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under Section 152 of the Act.

Section 2 Risk treatment measures for substantive risks

149. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people or the environment from the proposed field trial of GM chickpea. These risk scenarios were considered in the context of the scale of the proposed release (Chapter 1, Section 2.1), the proposed containment measures (Chapter 1, Section 2.2), and the receiving environment (Chapter 1, Section 5), and considering both the short and the long term. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

Section 3 General risk management

150. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and the environment are negligible. Therefore, to maintain the risk context, licence conditions have been imposed to limit the release to the proposed size, location and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment. The conditions are discussed and summarised in this Chapter and listed in full in the licence.

3.1 Draft licence conditions to limit and control the release

3.1.1 Consideration of limits and controls proposed by QUT

151. Sections 2.1 and 2.2 of Chapter 1 provide details of the limits and controls proposed by QUT in their application. Many of these are discussed in the three risk scenarios considered for the proposed release in Chapter 2. The appropriateness of these controls is considered further in the following sections.

152. The proposed release would take place at a single location at the Walkamin Research Facility, which is owned and managed by QDAF, in Walkamin, Qld. The trial would run for five and a half years (from June 2019 until December 2024), which could include up to six growing seasons. The maximum area planted would be three ha per season, with a single planting area planted each season. The small size and short duration of the trial would restrict the potential exposure of people and desirable animals to the GMOs (Risk Scenario 1).

153. The applicant proposes that only trained and authorised staff would be permitted to deal with the GMOs. Standard licence conditions included in the draft licence state that only people authorised by the licence holder are covered by the licence and that the licence holder must inform all people dealing with the GMOs of applicable licence conditions. These measures would limit the exposure of people to potential harm from the GM chickpea (Risk Scenario 1).

3.1.2 Consideration of proposed controls to manage exposure to the GMOs

154. The applicant proposes not allowing the GMOs or GM products to be used for human food or animal feed. A draft licence condition states that GM plant material must not be used as food for humans or feed for animals. This condition restricts the exposure of people and desirable animals to the GMOs (Risk Scenario 1).

155. The applicant has not proposed that the site would have fences with lockable gates, however, as the site is on a research station in a rural area it is not expected that persons other than those authorised under the licence would access the site. Standard conditions have been included in the draft licence that require that only authorised people are permitted to undertake any activity authorised by the licence and that all people dealing with the GMOs must be trained and informed of the relevant licence conditions. These measures are considered appropriate to limit the potential exposure of people to the GMOs (Risk Scenario 1) and would limit the opportunity for seed spread outside the trial area (Risk Scenario 2).

3.1.3 Consideration of proposed controls to manage pollen flow from the GMOs

156. The applicant has proposed a number of containment measures for the GM chickpeas, including the use of a 3 m monitoring zone and a 5 m isolation zone surrounding the planting area. They have also stated that no other chickpeas would be grown in these areas while the GM chickpeas are being grown. The potential for outcrossing of chickpeas has been discussed in Chapter 1 and in Risk Scenario 3. As noted there, consideration of outcrossing for this release is limited to other chickpeas as there are no related species present in Australia.

157. The available literature indicates that chickpea is almost entirely self-pollinating, with lower cross-pollination rates than a number of other crop species. Production of certified[6] seed for chickpeas are produced under various seed productions schemes, which specify, among other conditions, isolation requirements to ensure seed purity. The Seed Services Australia scheme requires an isolation distance of 3 m from other varieties of chickpeas (Seed Services Australia, 2013). In Canada an isolation distance of 1 m from other inspected pedigree chickpeas of the same variety or 3 m from other varieties of inspected pedigree chickpeas or non-pedigree chickpeas (CSGA, 2018), in California 10 ft (3.05 m) or a physical barrier including (but not limited to) a fence, ditch or bare ground (CCIA, 2015). The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) guidelines specify an isolation distance 10 m for Foundation seed or 5 m for Certified seed (Gaur et al., 2010).

158. Thus, the proposed licence conditions requiring a 3 m monitoring zone and a 5 m isolation zone where no chickpeas are grown , thus a total of 8 m isolation distance, is consistent with the available information about cross-pollination and with local and international requirements for producing pure seed. Although proposed by the applicant, there is no licence condition to prohibit planting of other chickpeas

[6] Different jurisdictions use different names for seed classes, - for simplicity the term ‘certified’ is used here to signify any class of seed which must be produced under a certification scheme.

outside this area, as it is considered that the proposed isolation distances are appropriate to manage risk of pollen transfer should another crop of chickpeas be planted.

159. The applicant has not proposed inspection conditions while the GMOs are being grown. However, draft licence conditions would require that the monitoring zone would be inspected for the presence of volunteers at least every 14 days from 28 days prior to expected flowering of the GMOs until 14 days after all GMOs have finished flowering. It is desirable to have one inspection after the completion of flowering of the GMOs, in case any plants were missed in the previous inspection, but no further inspections are necessary. Although the risk of outcrossing is minimal due to the absence of related species and the distance to any commercial chickpea production (Risk scenario 3), this requirement would ensure that the potential for spread of seed from the trial site is clearly understood and that risk management measures are appropriate to control any risk of seed spread (Risk Scenario 2).

3.1.4 Consideration of proposed controls to manage persistence of the GMOs

160. After harvest of each trial site, the applicant proposes to destroy all plant material from the trial not required for testing or future plantings. In order to manage persistence of GMOs, it is only necessary to destroy viable plant material, i.e. live GM plants or viable GM seed. Draft licence conditions require that the planting area must be cleaned (which would destroy any surviving GM plants) within 35 days after harvest, and that harvested GM seed not required to conduct experiments or for future planting, must be destroyed as soon as practicable.

161. The applicant proposes that any non-GM chickpeas planted as part of the trial would be treated as though they were GMOs. Non-GM chickpea grown at the trial site may be cross-pollinated by GM chickpea and bear hybrid seeds, although the outcrossing rates are very small. It is therefore appropriate to require non-GM chickpea to be treated in the same manner as GM chickpea, to manage persistence of the GMOs, and this measure is included in the draft licence. There are also conditions proposed in the draft licence that require that harvest of the GM chickpea be performed separately from any other crops.

162. The applicant has proposed that GM chickpea would be destroyed using one or more of the following methods: herbicide application, root cutting and mulching, hand weeding, autoclaving, destructive analysis or burial to a depth of at least 2 m. All of these methods are considered effective in destroying one or more life stages of the GM chickpea so are included in the draft licence. The applicant also proposed that the burial site would not be in a cultivatable area to mitigate the risk of disturbance and germination. However, given that chickpea seeds are unlikely to germinate and emerge successfully if planted too deep and that they do not tolerate waterlogged conditions (OGTR, 2019), it is considered that a burial depth of 1 m, with sufficient irrigation at the time of burial to encourage decomposition, is considered suitable to ensure the effectiveness of destruction of seed burial. Proposed licence conditions therefore require burial of seed to a depth of at least 1 m, with irrigation at the time of burial and specify that the burial site must not be intentionally disturbed for at least 12 months.

163. Following harvest, the applicant has proposed that the site would be inspected for chickpea volunteers at least every two months for at least 18 months and until the last six months are free of volunteers. Any volunteers found would be destroyed before flowering. Although there is strong observational information about chickpea persistence (OGTR, 2019), there is little documented evidence about how they may persist in different Australian environments. Additionally, this area is outside commercial chickpea production areas, so there a degree of uncertainty about how the conditions in this area may influence seed persistence. Thus, it was considered appropriate to propose a 24 month (two year) postharvest inspection period for this trial, to encompass two full growing seasons, with no volunteers detected for at least six months immediately prior to the end of the monitoring period.

164. The time from planting to flowering may vary across locations and different varieties, under Australian cropping conditions, chickpeas usually flower 90 - 110 days after sowing (GRDC, 2017b). Volunteer chickpea plants that grow during summer can flower earlier than chickpeas that are grown as a cool season crop in Australia, possibly as soon as 60 days after sowing (OGTR, 2019). The area in which the trial is located receives a high amount of rainfall following harvest, which would provide good soil moisture to promote germination of seeds in the soil or decomposition of seed on the surface. Thus it is likely that

seeds remaining at the trial site could readily germinate after harvest and that they may flower sooner than intentionally planted crops, a condition is proposed requiring post-harvest inspections should take place every 35 days.

165. The applicant has proposed that postharvest monitoring would include the planting area, monitoring and isolation zones, and any areas used to clean equipment or to bury seed. Conditions proposed in the draft licence require that the planting area and any areas outside the planting area where the GMOs have been dispersed in the course of dealings under this licence, must be cleaned as soon as practicable and before use for any other purpose. This would include the areas proposed by the applicant as well as any areas where seed may have been distributed, which is most likely during harvest and activates such as threshing. These conditions are considered suitable to manage risks associated with persistence of seeds at the trial site.

166. The applicant has proposed that any area used to bury seed as a means of destruction would be monitored for the presence of volunteers at least every two months for at least 18 months and until the last six months are free of volunteers. However, as volunteers are not expected to emerge on burial sites under normal circumstances, only monitoring for disturbance is proposed. This monitoring must be conducted for at least 12 months (the period during which the burial site must not be intentionally disturbed). However, if seed is dispersed during burial, or volunteers were observed during inspections for disturbance, this area would require cleaning as an area in which the GMOs have been dispersed in the course of dealings under the licence. In that case, post-cleaning conditions would apply.

167. The applicant has proposed that during the postharvest period the planting area would receive one shallow tillage when conditions are conducive to germination of volunteers and irrigation to encourage germination if soil moisture conditions were not sufficient for germination. As discussed in Chapter 1 and in Risk Scenario 2, chickpea seeds do not show dormancy. Chickpea seeds may survive for a few seasons if they are buried in dry soils or remain on the soil surface in dry conditions. However, if conditions are conducive to germination, chickpea seeds will germinate readily and thus viable seeds are unlikely to persist on the soil surface from season to season. Adequate soil moisture and seed-soil contact sufficient to access available soil moisture are necessary to provide conducive conditions (OGTR, 2019).

168. Shallow tillage and irrigation of the trial site during the postharvest period would promote suitable conditions for seed germination and for subsequent detection and destruction of volunteers, thus removing seed remaining at the site. Therefore, licence conditions have been proposed requiring shallow tillage and irrigation during the postharvest period in all areas that have been cleaned following harvest, with tillage occurring prior to the last irrigation. As the purpose of irrigation at that point is to provide adequate soil moisture for germination, the proposed conditions includes the provision for the licence holder to request that a natural rainfall event may be considered as equivalent to an irrigation. Evidence (such as rainfall measurements, photos of germinating plants etc.) that the rainfall has been sufficient to promote germination would be required.

3.1.5 Consideration of proposed controls to manage dispersal of the GMOs

169. The applicant has proposed that all equipment, including harvesters, seeders, storage equipment, transport equipment (e.g. bags, containers, trucks), tools, shoes and other clothing would be inspected for GM seeds and cleaned before using it for any other purpose. Such measures are considered appropriate to ensure seed is not unintentionally dispersed by equipment, so the draft licence contains a condition that requires any equipment used in connection with the GMOs must be cleaned as soon as practicable after use and before use for any other purpose. Requirements for cleaning of equipment associated with transport and storage of the GMOs would need to be conducted according to the requirements set out in the Regulators Guidelines for the Transport, Storage and Disposal of GMOs.

170. The applicant has proposed that a fence would be erected at the trial site to exclude feral pigs. They propose that the fence would be positioned around the edge of the isolation zone and moved if the size or

position of the planting area[7] varied from season to season. Feral pigs are present in the area and fencing is most effective if constructed before pigs have made pathways through the area that needs protection (QDAF, 2016). However, although feral pigs have been observed to eat germinating and emerging chickpeas and some seed may be present in faeces, it is unlikely that any seed consumed would remain viable (OGTR, 2019), thus it is unlikely that feral pigs would spread GM chickpea (Risk Scenario 2). Exclusion of large animals, including feral pigs and livestock may be achieved in a number of ways, including, but not limited to, the presence of a pig-proof fence. Therefore, the licence is not prescriptive in this regard.

171. Likewise, although other large animals including kangaroos, emus, turkeys (in central Qld) may consume chickpeas, it is unknown whether they can spread viable seed (OGTR, 2019), although unlikely given the size of the seed and associated risk of damage during ingestion and digestion (Risk Scenario 2). In addition, the lack of weedy chickpea populations around agricultural areas would indicate that any seed spread in such a manner is either not viable or is unable to survive outside cultivation. There is no reasonable expectation that GM chickpea would be more toxic to native fauna than non-GM chickpeas, so additional restriction of access by these animals would be necessary with respect to Risk Scenario 1.

172. Likewise, little is known about whether native or other birds consume and spread chickpea seeds. However, as discussed, the size of the seed would suggest that they would be damaged during ingestion and digestion, making the likelihood of spread of viable chickpeas by birds unlikely. Thus, it is considered unnecessary to impose additional measures to control access of birds to the planting area (Risk Scenario 2). Additionally there is no indication that the GM chickpeas wold be more toxic to birds than non-GM chickpeas, thus no restriction of access by birds is necessary in consideration of Risk Scenario 1.

173. The applicant has proposed the use of mouse baits, if required, to control rodents at the trial site. If this is to be an effective control measure, monitoring and detection of rodent activity would be needed in order to implement control measures at the required time. They have not indicated whether mice or other rodents are generally present at this location, however, as discussed in Risk Scenario 2, mice will consume germinating and emerging chickpeas (OGTR, 2019). Rodents are opportunistic feeders that will consume seeds and plant parts from crops (Caughley et al., 1998) and may move seeds from seed crops and hoard them (AGRI-FACTS, 2002), however the large size of chickpea seeds makes it unlikely that they would remove seeds from the trial site. If rodent baiting is to be used as a control measure it is more likely to be effective if used at all times when the GM chickpeas are growing. The applicant has also indicated that the monitoring and isolation zones would be maintained as bare fallow and this would provide conditions that do not attract or harbour rodents.

174. Recent licences for grain crop include conditions requiring the use of measures to control rodents in the planting area while GMOs are being grown and until the planting area has been cleaned. These measures include, but are not limited to, the use of rodent baiting or trapping. In addition, these licences include a condition which requires that the monitoring zone must be maintained in a manner that allows detection of chickpea volunteers and related species while the GMOs are being grown and until the area has been cleaned. Examples of this maintenance include keeping the monitoring zone free of vegetation, or planted with vegetation that is kept mown to a height of less than 10 cm. Such measures not only provide conditions suitable for detection of volunteers, but also provide conditions that do not attract or harbour rodents. These conditions are proposed in the draft licence to minimise the risks associated with rodent activity and to facilitate detection of GM plant material dispersed during dealings with the GMOs (Risk Scenario 2).

175. The applicant has proposed a distance of 100 m from the planting area to any natural waterway and selection of land not prone to flooding. These conditions would reduce the already small likelihood of any plant material, particularly chickpea seeds, being removed from the planting area by water (Risk Scenario 2) and have been included in the draft licence conditions. A condition has also been proposed requiring

[7] The applicant uses the term 'location' to indicate the area in which GM chickpeas are planted at the trial site

immediate notification of any extreme weather event affecting the trial site during the release to allow assessment and management of any risks.

176. As discussed in Risk Scenario 1, there is a very small possibility of hybridisation of different GM chickpea lines or hybridisation with non-GM chickpeas grown as part of the trial. However, any hybrids would not be expected to show different traits from either of the GM lines from which they were derived, not to produce any novel products, nor to show any difference in toxicity or allergenicity from either GM parent. In addition, given the proposed controls on access to the site and the post harvest monitoring requirements, it is unlikely that any hybrids would survive to produce seed (Risk Scenarios 1 and 2).

177. No information has been provided regarding the handling of seed immediately following harvest, although the applicant proposes that seed may be transported and used for experimental analysis in PC2 laboratories in Brisbane under appropriate Notifiable Low Risk Dealings (NLRDs) authorisation and may be used to plant further trials. Licence conditions specify that if seed harvested from the GMOs is threshed other than in accordance with NLRD requirements, it must be threshed separately from any other crop, and threshing must take place on a planting area or in a facility approved in writing by the Regulator.

178. The applicant has proposed that any GM plant material would be transported to approved facilities for analysis or destruction according to the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs. If seed required storage onsite before transport it would need to be stored according to the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs. Any grain remaining after analysis must be stored in an approved facility for subsequent use, or destroyed by autoclaving, burial or another method approved by the Regulator. These are standard conditions in the licence relating to the handling of GMOs, to minimise exposure of people and other desirable organisms to the GMOs (Risk Scenario 1), dispersal into the environment and gene flow (Risk Scenario 2 and 3).

3.1.6 Summary of draft licence conditions to be implemented to limit and control the release

179. A number of licence conditions have been drafted to limit and control the release, based on the above considerations. These include requirements to:

- limit the duration of the release to a maximum of six planting seasons, until December 2024
- limit the release to a single location in Qld – QDAF Walkamin Research Facility
- limit the release to a maximum total area of 3 ha per season
- locate trial sites at least 100 m from any natural waterways
- surround the planting area with a monitoring zone of at least 3 m, maintained in a manner that does not attract or harbour rodents, and in which volunteers must be prevented from flowering
- surround the monitoring zone with a 5 m isolation zone in which no chickpeas may be grown
- implement measures including rodent baits and/or traps to control rodents within the planting area
- harvest the GM chickpeas separately from other crops, using a dedicated plot harvester
- clean the areas after use including the planting area and any area in which seed has been dispersed
- clean any equipment used before use for any other purpose
- apply measures to promote the germination of any chickpea seeds that may be present in the soil after harvest, including irrigation and shallow tillage
- monitor for at least 24 months after harvest and destroy any chickpea plants that may grow and until no volunteers have been detected for a continuous six month period prior to the end of monitoring
- monitor any site used to bury seed for at least 12 months to detect any disturbance
- destroy all GMOs not required for further analysis or future trials
- transport and store the GMOs in accordance with the Regulator's guidelines
- not allow the GM plant material to be used for human food or animal feed

3.2 Other risk management considerations

180. All DIR licences issued by the Regulator contain a number of conditions that relate to general risk management. These include conditions relating to:

- applicant suitability
- contingency plans
- identification of the persons or classes of persons covered by the licence
- reporting requirements
- access for the purpose of monitoring for compliance.

3.2.1 Applicant suitability

181. 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, for either an individual applicant or a body corporate, include:

- any relevant convictions of the applicant
- 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 capacity of the applicant to meet the conditions of the licence.

182. If a licence were issued, the conditions would include a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

183. In addition, any applicant organisation must have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

3.2.2 Contingency plan

184. If a licence were issued, QUT would be required to submit a contingency plan to the Regulator before planting the GMOs. This plan would detail measures to be undertaken in the event of any unintended presence of the GM chickpea outside permitted areas.

185. Before planting the GMOs, QUT would also be required to provide the Regulator with a method to reliably and uniquely detect the GMOs or the presence of the genetic modifications in a recipient organism.

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

186. If a licence were issued, the persons covered by the licence would be 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. Prior to growing the GMOs, QUT would be required to provide a list of people and organisations that will be covered by the licence, or the function or position where names are not known at the time.

3.2.4 Reporting requirements

187. If issued, the licence would require 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.

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

- expected and actual dates of planting
- details of areas planted to the GMOs
- expected dates of flowering
- expected and actual dates of harvest and cleaning after harvest
- details of inspection activities.

3.2.5 Monitoring for compliance

189. 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.

190. 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.

191. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. The Act provides 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 4 **Issues to be addressed for future releases**

192. Additional information has been identified that may be required to assess an application for a commercial release of these GM chickpea lines, or to justify a reduction in limits and controls. This includes:

- additional molecular and biochemical characterisation of the GM chickpea lines, particularly with respect to potential for increased toxicity and allergenicity
- additional phenotypic characterisation of the GM chickpea lines, particularly with respect to increased abiotic or biotic stress tolerance or plant cell death that may contribute to weediness

Section 5 **Conclusions of the consultation RARMP**

193. The RARMP concludes that the proposed limited and controlled release of GM chickpea poses negligible risks to the health and safety of people or the environment as a result of gene technology, and that these negligible risks do not require specific risk treatment measures.

194. If a licence were issued, conditions would be imposed to limit the release to the proposed size, location and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment, as these were important considerations in establishing the context for assessing the risks.

Chapter 4 Proposed licence conditions

Section 1 **Interpretations and Definitions**

1. In this licence:

    a. unless defined otherwise, words and phrases used in this licence have the same meaning as they do in the Act and the Regulations;

    b. words importing a gender include any other gender;

    c. words in the singular include the plural and words in the plural include the singular;

    d. words importing persons include a partnership and a body whether corporate or otherwise;

    e. references to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;

    f. where any word or phrase is given a defined meaning, any other part of speech or other grammatical form in respect of that word has a corresponding meaning;

    g. specific conditions prevail over general conditions to the extent of any inconsistency.

2. In this licence:

'Act' means the *Gene Technology Act 2000* (Commonwealth) or the corresponding State legislation under which this licence is issued.

'Burial Site' means a place where the GMOs are Destroyed by burial.

'Chickpea' means plants of *Cicer arietinum* L.

'Clean' means, as the case requires:

a. in relation to Equipment or a Facility, remove and/or Destroy the GMOs; or

b. in relation to an area of land specified in this licence as requiring Cleaning:

    i. Destroy GM plants, if present, to the reasonable satisfaction of the Regulator, and

    ii. Thoroughly remove the GM seeds from the soil surface.

Note: One method of removing most GM seeds from the soil surface is Tillage, which moves seeds to under the soil.

'Contingency Plan' means a written plan detailing measures to be taken in the event of the unintended presence of the GMOs outside an area that must be inspected. A Contingency Plan must include procedures to:

a. ensure the Regulator is notified immediately if the licence holder becomes aware of the event; and

b. recover and/or Destroy the GMOs to the reasonable satisfaction of the Regulator; and

c. inspect for and Destroy any Volunteers that may exist as a result of the event to the reasonable satisfaction of the Regulator.

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

a. herbicide application;

b. root cutting and mulching;

c. hand weeding;

d. autoclaving;

e. destructive analysis;

f. burial, but only subject to the conditions of this licence; or

g. a method approved in writing by the Regulator.

Note: 'As the case requires' has the effect that, depending on the circumstances, one or more of these techniques may not be appropriate.

'Equipment' includes, but is not limited to, seeders, harvesters, threshers, storage equipment, transport equipment (e.g. bags, containers, trucks), clothing, footwear and tools.

'Facility' a facility approved in writing by the Regulator.

'Flowering' is taken to begin when any plant of the class of plants referred to in a particular condition first flowers, and is taken to end when all plants in the class of plants no longer have flowers.

'GM' means genetically modified.

'GMOs' means the genetically modified organisms that are the subject of the dealings authorised by this licence. GMOs include live plants and viable seed.

'Isolation Zone' means an area of land extending at least 5 m in all directions from the outer edge of the Monitoring Zone as indicated in Figure 1.

'Logbook' means a written or electronic record containing information required to be collected and maintained by this licence and which is able to be presented to the Regulator on request.

'Monitoring Zone' means an area of land extending outwards at least 3 m from the outer edge of the Planting Area, as indicated in Figure 1.

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

'Personal Information' means information or an opinion about an identified individual, or an individual who is reasonably identifiable:

a. whether the information or opinion is true or not; and

b. Whether the information or opinion is recorded in a material form or not

'Plant Material' means any part of the GM or non-GM Chickpea plants grown at a Planting Area, whether viable or not, or any product of these plants.

'Planting Area' means an area of land where the GMOs and non-GM Chickpea are planted and grown pursuant to this licence.

'Regulations' means the Gene Technology Regulations 2001.

'Regulator' means the Gene Technology Regulator.

'Sign-off' means a notice in writing from the Regulator, in respect of an area, that post-Cleaning obligations no longer apply in respect of that area.

'Site' means the area of land containing one Planting Area and associated Monitoring Zone. As shown in Figure 1.

'Tillage' means the use of any technique to disturb the soil.

'Volunteers' means GM or non-GM Chickpea plants which have not been intentionally grown.

'Waterways' means all permanent natural waterways and man-made waterways that flow into natural waterways.

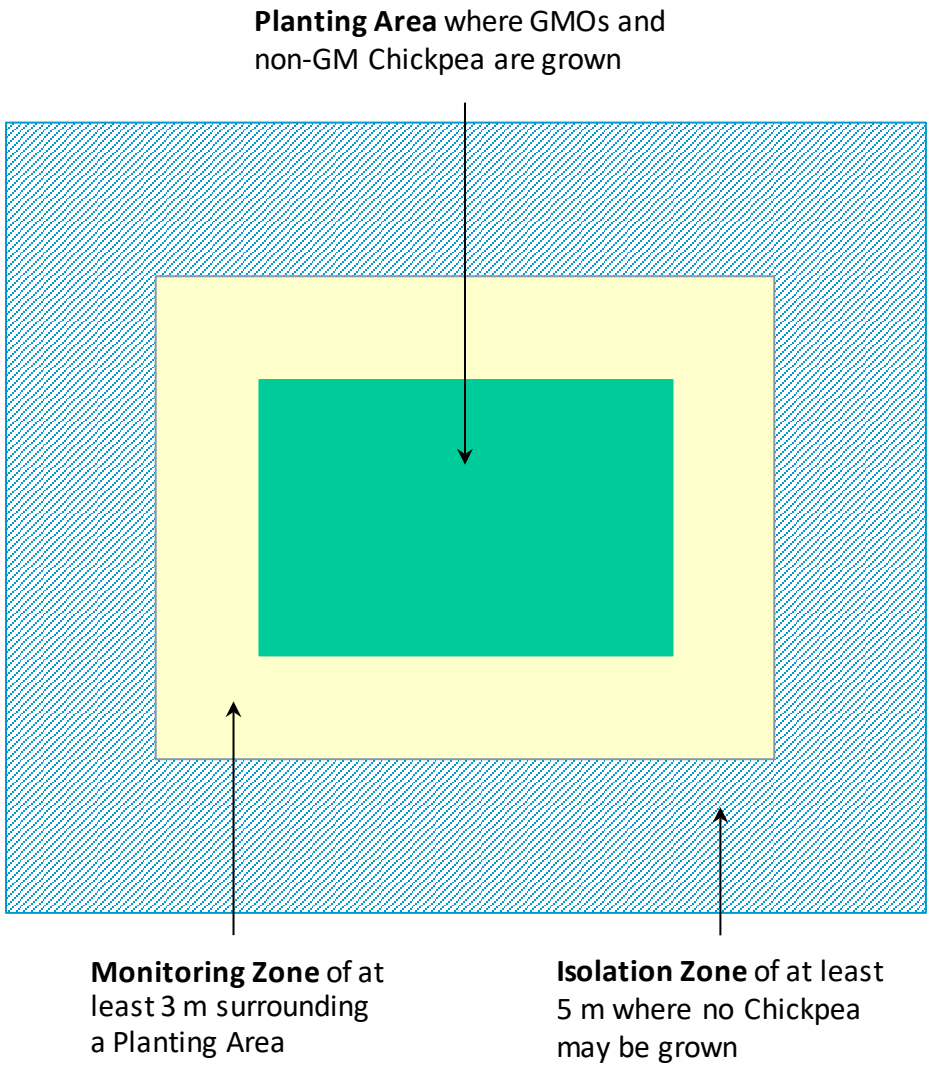

Figure 1 Diagram (not to scale) showing the relationship between Planting Area, Monitoring Zone, and Isolation Zone.

Section 2 General conditions and obligations

3. This licence does not authorise dealings with GMOs that are otherwise prohibited as a result of the operation of State legislation declaring an area as designated for the purpose of preserving the identity of GM crops, non-GM crops, or both GM crops and non-GM crops, for marketing purposes.

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

5. The licence holder is Queensland University of Technology.

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

7. The dealings authorised by this licence are to conduct experiments with the GMOs, breed, propagate, grow, transport and dispose of the GMOs, and possession, supply or use of the GMOs in the course of any of these dealings.

Obligations of the Licence Holder

8. The licence holder must notify the Regulator in writing as soon as practically possible if any of the contact details of the project supervisor change from that notified in the licence application or subsequently.

Note: please send all correspondence related to the licence to OGTR.M&C@health.gov.au.

Prior to issuing a licence, the Regulator considers suitability of the applicant to hold a licence. The following conditions address ongoing suitability of the licence holder.

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

10. The licence holder must:

a. inform the Regulator immediately in writing, of:

i. any relevant conviction of the licence holder occurring after the commencement of this licence; and

ii. any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment; and

iii. any event or circumstances occurring after the commencement of this licence that would affect the capacity of the holder of this licence to meet the conditions in it; and

b. provide any information related to the licence holder's ongoing suitability to hold a licence, if requested, within the stipulated timeframe.

11. The licence holder must be able to access and control the Planting Areas, Monitoring Zones, Isolation Zones, Burial Sites, areas used to Clean Equipment and approved Facilities to the extent necessary to comply with this licence, for the duration of the licence.

12. Prior to conducting any dealings with the GMOs, the licence holder must provide to the Regulator:

a. names of all organisations and persons or functions or positions of the persons who will be covered by the licence, with a description of their responsibilities; and

Note: Examples of functions or positions are 'project supervisor', site manager', 'farm labourer' etc.

b. detail of how the persons covered by the licence will be informed of licence conditions; and

c. detail of how the licence holder will access and control the Planting Area, Monitoring Zones, Isolation Zones, Burial Sites, areas used to Clean Equipment and approved Facilities, for the duration of the licence; and

Note: this may include a description of any contracts, agreements, or other enforceable arrangements.

d. written methodology to reliably detect the GMOs or the presence of the genetic modifications in a recipient organism. The detection method must be capable of identifying, each genetic modification event described in this licence; and

e. a Contingency Plan to respond to inadvertent presence of the GMOs outside an area that must be inspected.

13. Any changes to the information provided under the immediately preceding condition must be communicated in writing to the Regulator within 14 days of the changes occurring.

The following conditions seek to ensure that persons conducting the dealings are aware of the licence conditions and appropriate processes are in place to inform people of their obligations.

14. The licence holder must inform any person covered by this licence, to whom a particular condition of the licence applies, of the following:

    a. the particular condition (including any variations of it); and

    b. the cancellation or suspension of the licence; and

    c. the surrender of the licence.

15. The licence holder must not permit a person covered by this licence to conduct any dealing unless:

    a. the person has been informed of any applicable licence conditions, including any variation of them; and

    b. the licence holder has obtained from the person a signed and dated statement that the person:

        i. has been informed by the licence holder of the licence conditions including any variation of them; and

        ii. has understood and agreed to be bound by the licence conditions, or variation.

16. The licence holder must:

    a. inform the persons covered by this licence that any Personal Information relevant to the administration and/or enforcement of the licence may be released to the Regulator; and

    b. provide the Regulator, if requested, with copies of the signed and dated statements referred to in the immediately preceding condition.

Provision of new information to the Regulator

Licence conditions are based on the risk assessment and risk management plan developed in relation to the application using information available at the time of assessment. The following condition requires that any new information that may affect the risk assessment is communicated to the Regulator.

17. The licence holder must inform the Regulator if the licence holder becomes aware of:

    a. additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or

    b. any contraventions of the licence by a person covered by the licence; or

    c. any unintended effects of the dealings authorised by the licence.

Note: The Act requires, for the purposes of the above condition, that:

    a. *the licence holder will be taken to have become aware of additional information of a kind mentioned in Condition 17 if he or she was reckless as to whether such information existed; and*

    b. *the licence holder will be taken to have become aware of contraventions, or unintended effects, of a kind mentioned in Condition 17, if he or she was reckless as to whether such contraventions had occurred, or such unintended effects existed.*

Note: Contraventions of the licence may occur through the action or inaction of a person. For example if it is a condition of the licence that volunteers are destroyed prior to flowering and a volunteer flowers, then the person responsible for controlling volunteers will have contravened that licence condition.

18. If the licence holder is required to inform the Regulator under the immediately preceding condition, the Regulator must be informed without delay.

Note: An example of informing without delay is contact made within a day of the incident via the OGTR free call phone number 1800 181 030, which provides emergency numbers for incidents that occur out of business hours. Notification without delay will allow the OGTR to conduct a risk assessment on the incident and attend the location if required.

19. If the licence holder informs the Regulator under Condition 17 and the Regulator requests further information, such information must be provided in a manner, and within the time period, stipulated by the Regulator.

Obligations of persons covered by the licence

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

21. If a person is authorised by this licence to deal with the GMOs and a particular condition of this licence applies to the dealing by that person, the person must allow the Regulator, or a person authorised by the Regulator, to enter premises where the dealing is being undertaken, for the purposes of auditing or monitoring the dealing.

Section 3 **Limits and Control Measures**

3.1 Limits on the release

The following licence conditions maintain the risk assessment context within which the application was assessed, by imposing limits on where and when the GMOs may be grown, and on other activities that can be undertaken.

22. The only plants that may be intentionally grown at a Planting Area are:

    a. the GMOs covered by this licence as described in Attachment A of the licence;

    b. non-GM Chickpea plants; and

    c. plants approved in writing by the Regulator.

23. Non-GM chickpea plants grown in a Planting Area must be handled as if they were GMOs.

Note: Attachment A is not included in the draft licence as the plants are described in the Risk Assessment and Risk Management Plan.

24. Planting and growing of the GMOs may only occur within the following limits:

    a. between June 2019 and December 2024;

    b. at a single site: Queensland Department of Agriculture and Fisheries Walkamin Research Facility, Walkamin, Qld;

    c. a maximum area of 3 ha per year may be planted.

3.2 Control measures

The following licence conditions maintain the risk assessment context within which the application was assessed by restricting spread, persistence and exposure to the GMOs.

GMOs must not enter food or feed

25. Plant Material must not be used, sold or otherwise disposed of for any purpose which would involve or result in its use as food for humans or feed for animals.

Control measures related to pollen flow

26. The outer edge of the Planting Area must be surrounded by a Monitoring Zone (as indicated in Figure 1).

27. The Monitoring Zone must be maintained in a manner appropriate to allow the identification and/or destruction of Volunteers whilst the GMO is growing in the Planting Area until the Planting Area is Cleaned.

Note: Measures to achieve this could include areas of land free of any vegetation and/or vegetation kept mown to a height of less than 10 cm. Condition 57.d requires details of current land use and recent land management practices to be recorded upon inspection of the Monitoring Zone.

28. The Monitoring Zone must be surrounded by an Isolation Zone (as indicated in Figure 1).

29. The GMOs must not be grown in a Planting Area if any crop of Chickpea is present within the Monitoring or Isolation Zones.

30. While the GMOs are growing in a Planting Area, the Monitoring Zone must be inspected by people trained to recognise Chickpeas, and actions taken as follows:

Area	Period of inspection	Inspection frequency	Inspect for	Action
Monitoring Zone	**From** 28 days prior to the expected commencement of Flowering of any GMOs* **until** 14 days after all GMOs in the Planting Area have finished Flowering	At least once every 14 days	Volunteers	Destroy before Flowering or prevent from Flowering

**Condition 58.a.iv requires the licence holder to provide information to the Regulator on the expected flowering period, however the inspection period should be based on the observed development of the GMOs, so that inspections commence prior to flowering of any GMOs.*
Note: Details of any inspection activity must be recorded in a Logbook (Condition 57) and reported to the Regulator (Condition 58.f).

Control measures regarding seed dispersal

31. Any Equipment used in connection with the GMOs must be Cleaned as soon as practicable after use and before use for any other purpose.

32. Large animals, including feral pigs, must be excluded from the Site while GMOs are being grown on the Planting Area(s) and until the Planting Area(s) is Cleaned.

33. Measures must be implemented to control rodents within the Planting Area while GMOs are being grown and until the Planting Area has been Cleaned.

Note: Measures for rodent control may include, but are not limited to, traps and/or poison baits within and/or surrounding the Planting Area.

34. The Monitoring Zone must be maintained in a manner that does not attract or harbour rodents while the GMOs are being grown at a Planting Area and until the Planting Area is Cleaned.

Note: Measures to achieve this could include areas of land free of any vegetation and/or vegetation kept mown to a height of less than 10 centimetres.

35. The outer edge of the Planting Area must be at least 100 m away from Waterways.

36. The licence holder must notify the Regulator in writing as soon as reasonably practicable of any Extreme Weather event that could cause or has led to the dispersal of GMOs from a Planting Area while the GMOs are growing or from any area subject to Cleaning and post-Cleaning inspection requirements.

Note: The Contingency Plan must be implemented if the GMOs are detected outside areas under inspection (Condition 55).

37. The GMOs must be harvested separately from any other crop.

38. Harvesting must be conducted in a manner that avoids dispersal of GMOs outside the Planting Area.

Processing or experimentation with GMOs

39. If seed harvested from the GMOs is threshed other than in accordance with Notifiable Low Risk Dealings (NLRD) requirements, it must be threshed separately from any other crop, and threshing must take place on the Planting Areas or in a Facility approved in writing by the Regulator.

Note: Dealings conducted under an NLRD authorisation must be assessed by an Institutional Biosafety Committee before commencement, must comply with the requirements of the Regulations and are not subject to the conditions of this licence.

40. If processing of GM seed or experimentation, analysis or storage of the GMOs is not conducted in accordance with NLRD requirements, then such activities may only be undertaken within:

a. a Planting Area; or

b. a Facility approved in writing by the Regulator.

Note: Cleaning of a Facility must be reported to the Regulator (Condition 58.d).

41. Within a Facility approved under the preceding conditions, any area that is used for threshing, processing, experimentation or analysis of the GMOs must be Cleaned as soon as practicable and before use for any other purpose.

42. GMOs that are not required for further experiments or for future planting must be Destroyed as soon as practicable.

Transport or storage of the GMOs

43. If GMOs are stored prior to experimentation, they must be stored in a Facility within an unbreakable container labelled as containing GMOs.

44. If transport or storage of the GMOs is not conducted in accordance with NLRD requirements, such activities must:

a. only occur to the extent necessary to conduct the dealings permitted by this licence or other valid authorisation; and

b. be in accordance with the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs for PC2 GM plant as current at the time of transportation or storage; and

c. comply with all other conditions of this licence.

Note: Condition 15 requires signed statements for persons transporting or disposing of the GMOs.

45. Methods and procedures used to transport GMOs must be recorded, and must be provided to the Regulator, if requested.

Note: The Contingency Plan must be implemented if the GMOs are detected outside areas under inspection (Condition 55).

Destruction by burial

46. Burial must be conducted in a manner that minimises the likelihood of dispersal of the GMOs outside the Burial Site.

47. If Destruction of Plant Material occurs by burial:

a. The GMOs must be buried in a pit at Walkamin Research Facility and covered by a layer of soil at least 1 metre in depth, the top of which is no higher than the soil surface surrounding the burial site; and

b. Seeds must be sufficiently irrigated at the time of burial to encourage decomposition; and

c. within 14 days of burial, provide the Regulator a written notice indicating the precise location of the burial site (GPS coordinates and either a street address or other directions), the date on which burial occurred and broad description of the Plant Material buried (Planting Area and year the GMOs were planted); and

d. the Burial Site must not be intentionally disturbed for a period of at least 12 months from the date of burial; and

e. the Burial Site must be inspected during this period to identify any significant disturbance. If disturbance is identified, the licence holder must take appropriate remedial action and notify the Regulator of the disturbance and the remedial action taken.

Note: If Volunteers are observed on a Burial Site, the Burial Site becomes an area of land that requires Cleaning under Condition 51, and is subject to post-Cleaning requirements.
Note: Details of any inspection activity must be recorded in a Logbook (Condition 57) and reported to the Regulator (Condition 58.e).
Note: The licence cannot be surrendered until Burial Site conditions have been satisfied.

Cleaning

48. The Planting Area must be Cleaned within 35 days following harvesting of the GMOs.

49. If all GMOs in a Planting Area have been Destroyed, then for the purposes of this licence:

a. the GMOs are taken to have been harvested; and

b. the Planting Area is taken to have been Cleaned.

Note: Cleaning activities must be reported to the Regulator (Condition 58.d)

50. Any area outside the Planting Area where the GMOs have been dispersed in the course of dealings under this licence, must be Cleaned as soon as practicable and before use for any other purpose.

Notes: This would include, but is not limited to approved Facilities, areas used to Clean Equipment.

51. Areas of land and Equipment used in connection with the GMOs must be Cleaned as follows:

Areas/Equipment to be Cleaned	When
a. Planting Area	35 days after harvest of the GMOs
b. any area where GMOs have dispersed during planting, growing or harvesting c. any Equipment used in connection with the GMOs d. any area used to Clean any Equipment used in connection with the GMOs e. any area used to experiment with, analyse or store GMOs	As soon as practicable and before use for any other purpose

Notes: Cleaning activities must be reported to the Regulator (Condition 58.d). Areas of land that have been Cleaned, or from which the GMOs have been harvested, are also subject to Inspections (Condition 52).

Post-Cleaning requirements

52. Post-Cleaning areas of land must be inspected by people trained to recognise Chickpea. Inspections must cover the entirety of the areas to be inspected. Actions must be taken as follows:

Area of land	Period of inspection	Inspection frequency	Inspect for	Action
Planting Area or other areas that have been Cleaned	From the day of completion of Cleaning of the Planting Area, until: i. the area is replanted with the GMOs; or ii. the Regulator has issued a Sign-off for the area.	At least once every 35 days	Volunteers	Destroy before Flowering

53. While post-Cleaning inspection requirements apply to the Planting Area:

a. the area must be maintained in a manner appropriate to allow identification of Volunteers; and

b. no plants may intentionally be grown in the area unless the plants are:

i. the GMOs or non-GM Chickpea planted in accordance with the conditions of this licence; or

ii. agreed to in writing by the Regulator; and

c. prior to the final irrigation referred to in Condition 53.e, the area must be Tilled at a time that would promote the germination of Volunteers within the volunteer-free period immediately prior to the Sign off application; and

d. any Tillage of the area must be to a depth no greater than the depth of sowing of the GMOs; and

e. prior to an application for Sign-off, the area must receive at least three irrigations, at intervals of at least 28 days, with the last required irrigation occurring at a time that would promote the germination of Volunteers within the volunteer-free period immediately prior to the Sign-off application.

Note: A period of natural rainfall (as recorded in condition 57.f) may be taken as irrigation only with the agreement of the Regulator. Evidence (such as rainfall measurements, photos of germinating plants etc.) that the rainfall has been sufficient to promote germination should be provided.

54. For a Facility, once Cleaning has been completed, the licence holder must notify the Regulator that the Facility has been Cleaned.

Contingency plan

55. If any unintentional presence of the GMOs is detected outside the areas requiring inspection, the Contingency Plan must be implemented.

Section 4 **Sign off**

56. The licence holder may make written application to the Regulator that planting restrictions and inspection requirements no longer apply to the Planting Area and other areas requiring Cleaning if:

a. all post-Cleaning inspection activities have been conducted for at least 24 months on the area; and

b. conditions have been conducive for germination and detection; and

c. no Volunteers have been detected on this area for at least six months of the inspection period immediately prior to the Sign off request.

Note: The Regulator will take into account the management and inspection history for the Planting Area and associated areas, including post-harvest crops planted (if any), Tillage, irrigation, rainfall, application of herbicide and occurrence of volunteers, in deciding whether or not further inspections are required to manage persistence of the GMOs.

Section 5 **Reporting and Documentation**

The following licence conditions are imposed to demonstrate compliance with other conditions, facilitate monitoring of compliance by staff of the OGTR, and emphasise appropriate selection of the Planting Area.

57. Details of any inspection activity must be recorded in a Logbook and must include:

a. date of the inspections;

b. name of the person(s) conducting the inspections;

c. details of the experience, training or qualification that enables the person(s) to recognise Volunteers, if not already recorded in the logbook;

d. details of areas inspected including current land use and recent management practices applied;

Note: management practices includes Tillage events, spraying or maintenance measures used to facilitate inspections

e. details of the developmental stage of the GMOs while they are being grown;

f. details of any post-harvest rainfall events including measurements at or near the area, or any irrigation events;

g. details of any Volunteers observed during inspections or during land-management activities, including number, developmental stage and approximate position of the Volunteers within each area inspected[†];

h. date(s) and method(s) of Destruction of or preventing Flowering of any Volunteers, including destruction of Volunteers during land-management activities; and

i. details of rodent control methods used and any evidence of rodent activity.

[†] *Examples of acceptable ways to record the positional information for Volunteers in the Logbook include:*
- descriptive text
- marking on a diagram
- indicating grid references on corresponding map/sketch

Note: Details of Inspection activities must be provided to the Regulator (Condition 58).The Regulator has developed a standardised proforma for recording inspection activities. This can be made available on request.

58. Notifications must be sent to the Regulator as follows:

Notice	Content of notice	Timeframe
a. Intention to Plant	i. Details of the Planting Area including size, the local government area, GPS coordinates, a street address, a diagrammatical representation of the trial sites (e.g. Google Maps) and any other descriptions. ii. Identity of the GMOs to be planted at the Planting Area (e.g. lines or construct details) iii. Date on which the GMOs will be planted iv. Period when the GMOs are expected to Flower v. Period when harvesting is expected to commence vi. How all areas requiring post-Cleaning inspections are intended to be used until sign-off, including the proposed post-harvest crop(s) (if any) vii. Details of how you propose to manage inspection activities, including strategies for the detection and destruction of volunteer GMOs viii. History of how the site has been used for the previous two years	At least 7 days prior to each planting (to be updated immediately if the notified details change)
b. Planting	i. Actual date(s) of planting the GMOs ii. Any changes to the details provided under part (a) of this condition iii. Details of all sexually compatible lines being grown concurrently under any other licence.	Within 7 days of any planting
c. Harvest	i. Actual date(s) of harvesting the GMOs	Within 7 days of commencement of any harvesting
d. Cleaning	i. Actual date(s) on which any areas needing Cleaning were Cleaned ii. Method of Cleaning	Within 7 days of completion of any Cleaning
e. Burial	i. Actual date(s) and precise location of Burial ii. Broad description of the GMOs buried (Condition 47.c) iii. Record of any disturbance to the Burial Site and remedial actions taken iv. Record of any Volunteers observed at the Burial site and details of Destruction	Within 14 days of any burial As soon as practicable Within 7 days of completion of any Cleaning
f. Inspection activities	i. Information recorded in a Logbook as per the inspection requirements (Conditions 30, 52 and 57).	Within 35 days of Inspection

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