Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 189

Limited and controlled release of sorghum genetically modified for asexual seed formation

Applicant: The University of Queensland

June 2022

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Summary of the Risk Assessment and Risk Management Plan for Licence Application No. DIR 189

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application for the intentional release of a genetically modified organism (GMO) into the environment. A Risk Assessment and Risk Management Plan (RARMP) for this application has been prepared by the Regulator in accordance with the *Gene Technology Act 2000* (the Act) and corresponding state and territory legislation, and finalised following consultation with a wide range of experts, agencies and authorities, and the public. The RARMP concluded that the proposed field trial poses negligible risks to human health and safety and the environment and that any risks posed by the dealings can be managed by imposing conditions on the release.

The application

Project Title	Limited and controlled release of sorghum genetically modified for asexual seed formation[1]
Parent organism	Sorghum (*Sorghum bicolor*)
Genetic modifications	
Introduced genes and modified traits:	Expression of a grass gene[2] involved in altering the reproduction mode of sorghum from sexual to asexual
Genetic modification method	*Agrobacterium*-mediated
Number of lines	Up to 10 independent lines of a number of sorghum cultivars
Principal purpose	To assess agronomic characteristics, seed viability, gene persistence, yield and yield components, and grain quality of the GM sorghum plants under field conditions
Previous releases	There have been no previous releases of the GMOs
Proposed limits	
Proposed use of GM plants	No use in human food or animal feed is proposed
Proposed location	University of Queensland's Gatton Campus - Crop Research Unit
Proposed release size	Up to 1 ha per year
Proposed period of release	From September 2022 to June 2025

[1] The title of the project as supplied by the applicant is 'Limited and controlled release of *Sorghum bicolor* genetically modified for altered reproduction from sexual to asexual'

[2] Confidential Commercial Information: The details of the introduced gene have been declared as Confidential Commercial Information under section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

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 risks 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, and transfer of the introduced genetic material to non-GM sorghum 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 proposed limits and controls, such as not using GM plant material in food or animal feed, will effectively minimise exposure to the GMOs. In addition, there is no evidence to suggest the introduced genetic modifications would lead to harm to people or the environment.

Risk management plan

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.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the 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 the GMOs are destroyed.

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Abbreviations

°C	Degrees Celsius
CCI	Confidential Commercial Information
cv.	cultivar
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GMO	Genetically modified organism
IBC	Institutional Biosafety Committee
MiMe	Phenotype where meiosis is turned into mitosis
NLRD	Notifiable Low Risk Dealings
OGTR	Office of the Gene Technology Regulator
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
spp.	species
subsp.	subspecies
the Act	*Gene Technology Act 2000*
UQ	The University of Queensland

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. 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, Water and the Environment. Proposed dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

6. 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.

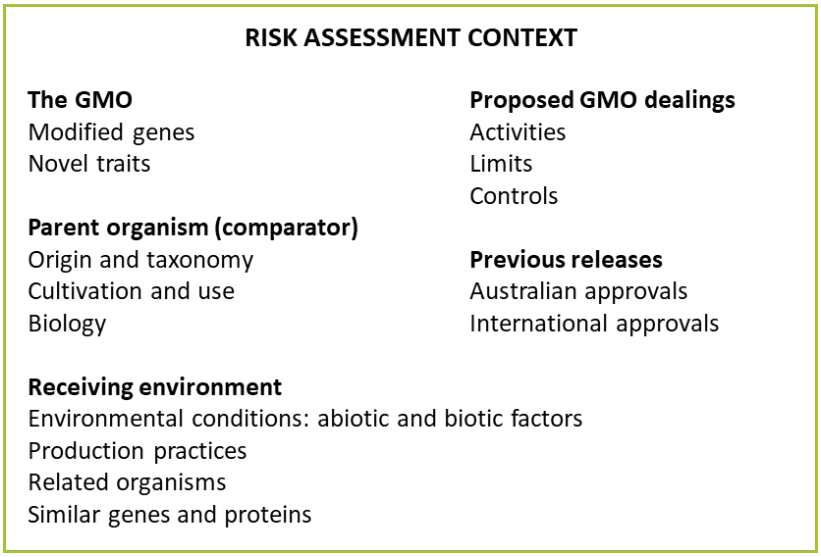

Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the Risk Analysis Framework.

7. Section 52 of the Act requires the Regulator to seek comment on the RARMP from agencies - the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, Australian local councils, and the Minister for the Environment - and from the public. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. Two public submissions were received and their considerations are summarised in Appendix B.

Section 2 The proposed dealings

8. The University of Queensland proposes to release up to 10 genetically modified (GM) sorghum lines into the environment under limited and controlled conditions. The GM lines have been genetically modified to alter its reproduction pathway. The introduced gene is one component (of several) involved in allowing sorghum to reproduce asexually rather than sexually. This DIR application does not propose to modify the sorghum to include the addition of all components needed to fulfill asexual reproduction (see Section 2.3.1)

9. The purpose of the trial is to evaluate the agronomic characteristics, seed viability, gene persistence, yield and yield components and grain quality of the GM sorghum plants under field conditions. The GM sorghum lines would not be used for human food or animal feed.

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

- conduct experiments with the GMOs
- breed the GMOs
- propagate the GMOs
- grow or culture the GMOs
- import the GMOs
- transport the GMOs
- dispose the GMOs

and the possession, supply or use of the GMOs in the course of any of these dealings.

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

11. The release is proposed to take place at one site at the University of Queensland's Gatton Campus (Lockyer Valley LGA in Queensland). The release is proposed to take place between September 2022 and June 2025. Up to one hectare would be grown on this site per season.

12. Only trained and authorised staff would be permitted to deal with the GM sorghum.

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

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

- transport, storage and disposal of the GM sorghum would be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*
- GM and non-GM sorghum from the trial and its products would not be used for animal feed or human food
- non-GM plants used and produced in the trial would be treated as if they were GM
- harvesting of sorghum heads would occur by hand or with commercial equipment. If used, all commercial equipment would be cleaned in accordance with the Regulator's requirements.
- all other equipment, including clothing, would be inspected for sorghum seeds and cleaned before use for any other purpose, or removal from the site

- all people conducting dealings with the GM sorghum would be trained with the licence conditions if issued
- all sorghum heads would be bagged to support self-pollination and limit pollen spread. The bags would be removed after pollen shed has ceased and pollen grains are no longer viable
- cultivated sorghum and related species would be excluded from a 100 m zone around the field trial site, and the planting date of GM sorghum adjusted to limit synchronous flowering of non-GM sorghum within 300 m of the trial
- the field site would be a minimum of 100 m from a natural waterway
- the GM sorghum would be planted under a bird and hail-proof enclosure (hail guard netting) and this would also exclude larger animals
- baiting would reduce the number of small rodents eating the grain
- post-harvest monitoring every 35 days would identify any volunteer plants allowing them to be removed and destroyed.

14. The proposed limits and controls are taken into account in the risk assessment (Chapter 2) and their suitability for containing the release is evaluated in the risk management plan (Chapter 3).

2.3 Details of the proposed dealings

2.3.1 Summary of the proposed genetic modification

15. Hybrid vigour or heterosis occurs when the offspring of sexually reproducing parents display enhanced traits when compared to either of its parents. These enhanced traits are not 'fixed' due to genetic segregation in the offspring during its own sexual reproduction. Therefore, hybrid vigour needs to be recreated each generation by crossing the original parents. A potential means to 'fix' these enhanced traits would be of advantage to maintain improved lines and could occur through the offspring reproducing asexually.

16. In plants, asexual seed reproduction is termed apomixis. It occurs naturally in various plant species, e.g. in meadow grasses (Poa spp.), brambles (Rubus spp.) and dandelions (Taraxacum spp.). In sorghum, induction of the asexual mode of seed formation would require changes in three key developmental stages (Figure 2).

- First, the sexual reproduction where the mixing and halving of genetic material from the parent plant/plants known as 'meiosis' needs to stop. Instead of halving, these cells need to duplicate identically by the process known as 'mitosis'. This change in the reproductive pathway can be brought about by mutation in three genes turning meiosis into mitosis (*MiMe*). The genes involved in the *MiMe* phenotype are conserved in plants (d'Erfurth et al., 2009). This means the male and female reproductive cells are genetically identical to the parent plant. The applicant is not proposing to introduce changes to the *MiMe* phenotype in the GM sorghum proposed for this release.
- The initiation of the embryo component of the seed now needs to occur without fertilisation in a process known as parthenogenesis. This step is targeted in the current proposal via introduction of the parthenogenesis gene into sorghum.
- Finally, the development of the endosperm component of the seed, critical for seed germination, commercial food and feed uses and grain quality needs to occur efficiently with or without fertilisation. The applicant is not proposing to introduce changes to this trait into the GM sorghum in the current proposal.

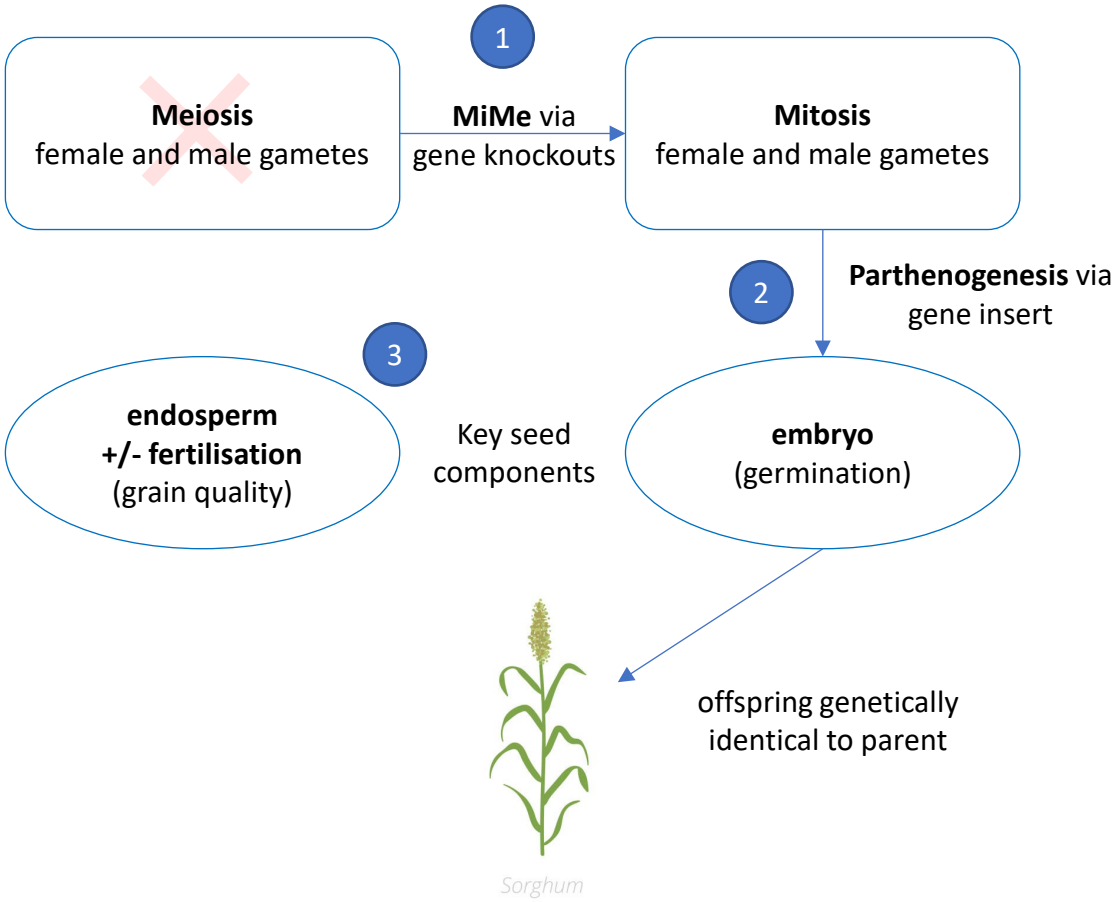

Figure 2. A graphical representation of the three key developmental stages needed to produce asexual sorghum. The applicant is proposing to only modify the plant at stage 2 under this DIR. Stages 1 and 3 would not take place. Figure adapted from that provided by the applicant.

17. From the three key developmental stages (Figure 2), the applicant is only proposing to produce GM sorghum plants by introducing a gene to initiate parthenogenesis. As such, the GM sorghum plants would still undergo sexual reproduction as meiosis is not impaired and no genetic modifications to allow endosperm development without fertilisation is proposed.

2.3.2 Development of the GM sorghum

18. Three lines of the GM sorghum (RTx430) have been developed by UQ's collaborators overseas and have been imported into Australia. UQ would then develop seven further lines. Each line is considered a single independent transformation event and each line would contain a single copy of the introduced gene. The introduced gene would remain under Mendelian segregation as meiosis during male and female gametogenesis would remain unchanged.

19. Several diverse sorghum backgrounds containing the gene insertion would be tested to determine its stability across different genotypes. These GM sorghum lines would be produced in one of two ways: (1) by gene insertion via *Agrobacterium*-mediated transformation[3]; or (2) by manual cross-pollination, conducted in a controlled glasshouse under an NLRD, from a GM sorghum to wild-type sorghum. The resulting GM sorghum lines from cross-pollination and their subsequent progeny are to be considered the same line from the original transformation event. Thus, the number of lines released, defined as 'encompassing all progeny from a single transformation event' would not exceed ten independent lines.

[3] Information about these methods can be found in the document *Methods of plant genetic modification*, available from the OGTR Risk Assessment References page.

20. Potential diverse grain sorghums of the same species from different backgrounds include the races: durra, kafir, guinea, caudatum and other improved lines.

Section 3 Parent organism – *Sorghum bicolor*

21. The parent organism is cultivated grain sorghum (*Sorghum bicolor* (L.) Moench subsp. *bicolor*). *S. bicolor* is exotic to Australia and its distribution in the country is widespread. However, it is cultivated extensively in Qld and NSW as an important summer crop, with an average of over 600,000 ha/year planted in recent years (GRDC, 2017; OGTR, 2017; ABARES, 2021). Within Australia, sorghum is used predominantly as livestock feed but also for production of ethanol fuel (Grain Growers, 2021). Sorghum flour is also used in some gluten-free food products in Australia (GRDC GroundCover 2019). Grain is exported for both animal and human consumption (Grain Growers, 2021). The GM sorghum from this DIR trial would not be used for human or animal consumption.

22. Detailed information about sorghum is contained in the reference document produced to inform the risk analysis process for licence applications involving GM crops: The Biology of *Sorghum bicolor* (L.) Moench subsp. *bicolor* (Sorghum) (OGTR, 2017). Aspects of sorghum's biology has also been described in the RARMP for DIR-153 (Limited and controlled release of sorghum genetically modified for grain quality traits). Baseline information from these documents will be used and referred to throughout the RARMP. Key points from those discussions are summarised as necessary in this RARMP.

23. The predominant GM sorghum cultivar that would be genetically modified is RTx430. As described in the RARMP for DIR-153, RTx430, an American inbred cultivar that is commonly used as a male parent in sorghum hybrid breeding worldwide. The average height of RTx430 sorghum plants is 102-107 cm, and the average time from planting to 50% flowering is 78 days in field trials.

24. Sorghum grain is a staple food in many countries in Africa and Asia and is grown for livestock feed in Australia and other developed countries (OGTR, 2017). The proteins in sorghum grain are regularly consumed by humans and livestock without adverse effects. However, non-GM sorghum contains a number of toxins (including those produced by various fungi growing on sorghum), anti-nutritional factors and allergens that, in extreme cases, may have toxic and allergenic effects (OGTR, 2017).

25. Some of the Australian grain sorghum crop is exported for human consumption, particularly for production of baijiu, a traditional Chinese distilled liquor (Grain Growers, 2021).

Section 4 The GMO – nature and effect of the genetic modification

26. Parthenogenesis is the spontaneous development of an embryo from an unfertilised egg cell: parthenos = virgin, genesis = creation. It naturally occurs in a variety of plant and animal species. In plants, parthenogenesis is usually found in combination with apomeiosis (the omission of meiosis) and pseudogamous or autonomous (with or without central cell fertilisation) endosperm formation. Together, parthenogenesis and apomeiosis is known as apomixis (clonal seed production) (Vijverberg et al., 2019).

27. In plants, the egg cell after undergoing meiosis and containing a haploid genome (1n) develops within a female gametophyte. Fertilisation of the egg cell with a haploid male sperm cell results in a diploid (2n) genome. Parthenogenesis usually occurs in combination with a mechanism that keeps or restores the diploid chromosome number, since haploid offspring are usually less fit or nonviable in nature. See Vijverberg et al. (2019) for a review of the various mechanisms involved.

28. The success of embryo development also relies on the nutrition of the embryo, which in angiosperms (flowering plants) is provided by the endosperm. However, the endosperm, in sexually reproducing plants, itself requires fertilisation of the central cell. The process of double fertilisation in which the egg cell and central cell are each fertilised by one of two clonal sperm cells is unique to flowering plants, reviewed in Vijverberg et al. (2019). In most apomictic species, endosperm development is pseudogamous, requiring fertilisation of the central cell, whereas in a minority of apomictic species, the endosperm develops autonomously (Vijverberg et al., 2019).

29. As stated in Paragraph 15, harnessing asexual reproduction (or apomixis) in plant breeding would enable rapid fixation of traits in hybrid crop plants. The applicant aims to explore the parthenogenic component of apomixis by introducing, into sorghum, a parthenogenic gene by way of genetic modification.

4.1 The introduced parthenogenesis gene

30. The sorghum plants intended to be used in this field trial have been genetically modified by insertion of a parthenogenesis gene. Further information regarding the identity and source of this gene and its performance in other transgenic plants, and the identities and sources of associated regulatory elements has been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

4.1.1 Inheritance of the introduced gene and anticipated changes to the phenotype in progeny

31. The introduced parthenogenesis gene would be under the transcriptional control of tissue-specific promoters. As no genetic modifications to stop meiosis are proposed, the GM sorghum would undergo meiosis during male and female gametogenesis. The following paragraphs describe the anticipated phenotype in progeny resulting from sexual reproduction of the diploid GM parents where each parent is hemizygous for the genetic modification and contains a single copy of the parthenogenesis gene. This prediction is based on preliminary results reported by the applicant that were obtained under glasshouse conditions and assumes that the introduced gene offers no selective advantage to the GM sorghum, that meiosis occurs, and that the introduced gene segregates under Mendelian inheritance. Figure 3A-C illustrates the genetic outcomes where the introduced gene is expressed, and its gene product is active, imparting parthenogenesis; Figure 3D illustrates the genetic outcomes if the introduced gene is inactive, and parthenogenesis does not occur.

32. If the introduced gene is expressed, then the following is expected:

- In half the progeny, i.e. the progeny from female gametes into which the introduced gene has segregated, sperm-mediated fertilisation of the egg cell is not required as an embryo would develop parthenogenetically. GM sorghum developing from those seeds would be haploid, weak and not be able to produce seed (Figure 3A). The applicant has conducted preliminary glasshouse trials overseas where they have observed these plants not to produce seeds and to be one-third shorter than the GM parent and non-GM sorghum. As no further offspring is produced, the introduced gene would not remain in the environment.

- One quarter of the progeny would be diploid, fertile and very similar to the parental GM sorghum as they would contain one copy of the introduced gene (Figure 3B). This outcome would come about when a female egg, not containing the introduced gene, is fertilised by a male gamete containing the introduced gene. This progeny would be able to pass the introduced gene to its offspring in the manner illustrated in Figure 3A-C, i.e. a quarter of its offspring will continue to pass on the introduced parthenogenesis gene into the next generation, and so on.

- The remaining quarter of the progeny would not inherit the introduced gene (Figure 3C). This progeny would be diploid, fertile, and non-GM, and go on to produce diploid, fertile non-GM seeds.

33. It is possible that the introduced gene does not impart parthenogenesis in the female gamete, e.g. due to a cellular condition preventing expression of the introduced gene or activity of the gene

product in the egg cell. Depending on whether or not the female gamete and the fertilising male gamete contain the introduced gene, the resulting embryo could be either homozygous or hemizygous for the introduced gene, or not inherit the gene (Figure 3D). Any seeds could develop into diploid, fertile sorghum. These would not display the parthenogenesis phenotype, irrespective of whether or not they contain the introduced gene. Where the introduced gene is present, it would be passed down to further generations in a Mendelian fashion. Over generations, the introduced trait may or may not become active due to natural mutation or restoration of expression.

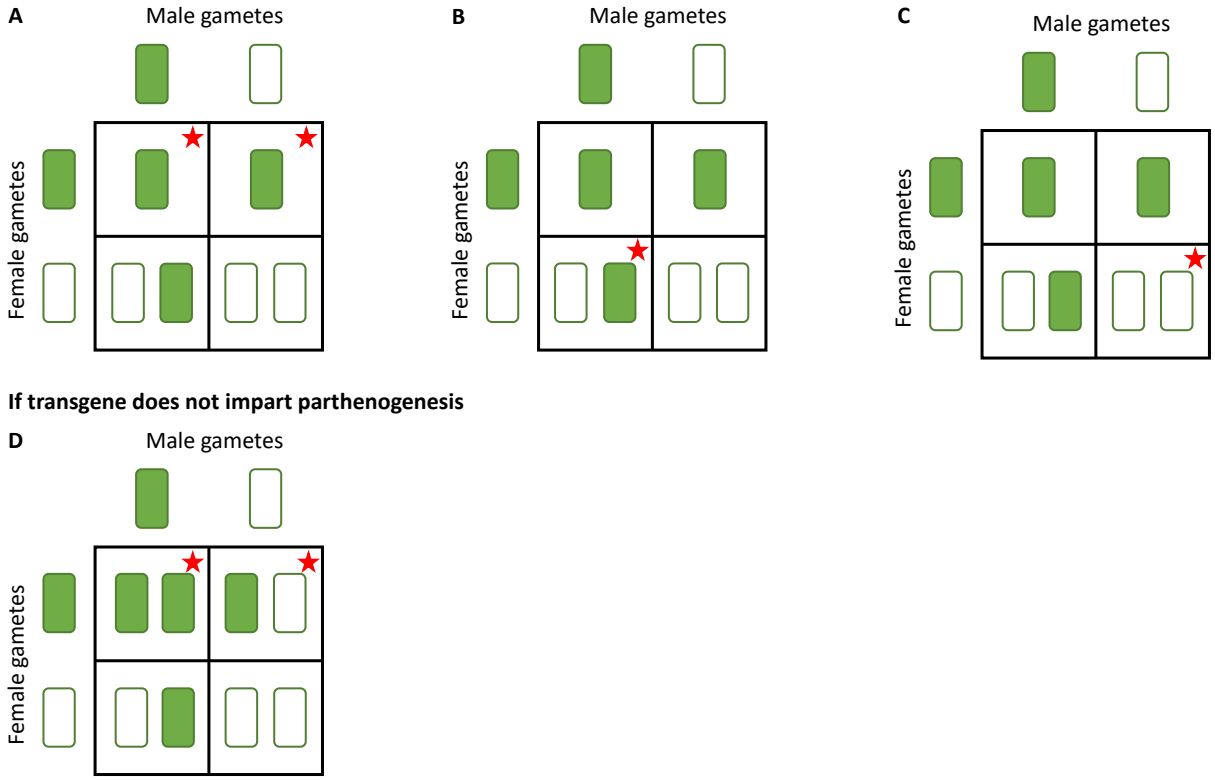

Figure 3. Expected outcomes of progeny arising from sexual reproduction between diploid GM sorghum carrying a single copy of the introduced gene. Note all percentages are based on theoretical approximates. (A) 50% of the progeny seed would contain a haploid genome as parthenogenesis would take place and the male gamete would not contribute to the genome. (B) 25% of the progeny seed would result in a genotype that is very similar to the diploid GM parental sorghum. (C) 25% of progeny seed would be null diploid as they would not have inherited the introduced gene from either the male or female gamete. (D) If the introduced gene is inherited in the female gamete, but is not active, then the gamete cannot undergo parthenogenesis. After fertilisation, one or two copies of the introduced gene would be present in the progeny seed without contributing parthenogenesis. Key: green locus = introduced parthenogenesis gene; white locus = null for introduced parthenogenesis gene; red star = highlighting the punnet square(s) in relation to the description.

4.1.2 Adverse effects associated with the introduced gene

34. A comprehensive search of the scientific literature yielded no information to suggest that the introduced gene or its protein product have toxic or allergenic effects to people or other organisms.

35. The introduced gene specifically alters cell-specific reproductive pathways by inducing parthenogenesis and somatic embryogenesis, and it is not known to confer any other phenotypic changes. Orthologues of the introduced gene are present in the genomes of many grass species, including major crops such as maize and rice.

Section 5 The receiving environment

36. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. It informs the consideration of potential exposure pathways, including the likelihood of the GMOs spreading or persisting outside the site of release. 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).

37. Information relevant to the growth and distribution of commercial sorghum in Australia is discussed in *The Biology of Sorghum bicolor (L.) Moench subsp. bicolor (Sorghum)* (OGTR, 2017).

5.1 Relevant abiotic factors

38. The release is proposed to take place at the University of Queensland Gatton Campus. The average temperatures in Gatton range between 17.5 - 32.5°C in summer and 6.5 - 23.5°C in winter (Bureau of Meteorology website; data range from 1991-2021). The predominant GM sorghum cultivar that would be genetically modified is RTx430. As stated in the RARMP for DIR-153, RTx430 is photoperiod insensitive[4] (Cuevas et al., 2016) so in principle could grow throughout the year. However, RTx430 has low cold tolerance and seeds germinate poorly at soil temperatures lower than 16°C (Franks et al., 2006). Thus, the GM sorghum would not be expected to grow well during winter in south-east Queensland. Sorghum is most sensitive to heat stress during flowering, as high daily temperatures (e.g. 36°C during the day and 26°C at night or 38°C during the day and 21°C at night) during this period will reduce seed set (Nguyen et al., 2013). These temperatures are higher than the average summer temperatures in south-east Queensland, but the GM sorghum could suffer from heat stress if a heat wave coincided with flowering.

39. Modern sorghum cultivars are also photoperiod insensitive, and this trait is likely to be present in the other improved sorghum lines proposed to be used in the release. Photoperiod-sensitive cultivars, if released, would only be able to flower in short day lengths of 12 hr or less (Higgins et al., 2014) and therefore should not flower in summer months in Queensland, where the days are longer than 12 hrs.

40. Sorghum is a water-efficient crop with high drought tolerance (GRDC, 2017). It is estimated that over 90% of sorghum in Australia is grown as a dryland crop (Philp et al., 2010). The applicant proposes that the GM sorghum would be grown either on irrigated or dryland areas within the trial site.

41. South-east Queensland experiences significant rainfall events that may lead to severe flooding every 10-20 years, and with climate-change the frequency is increasing. In recent history, there has been major widespread flooding in 1974, 2011, and more recently in February/March 2022. The application for DIR-189 was received in November 2021 and the applicant stated that the Gatton site did not flood during the peak flood years of 1974 or 2011 nor any other recorded time. After the floods in February/March 2022, the applicant informed the OGTR that the Gatton site was affected . OGTR staff then visited the proposed field trial site and found that although parts of the Gatton campus were affected by the floods, including areas surrounding the planting area, the proposed planting area itself was not flooded. It did, however, get very wet. Although this single flood event does not imply that the Gatton site is in a flood prone area, the possibility of major flooding in upcoming years at this site cannot be discounted.

[4] Photoperiod sensitivity: ability of a plant to detect and respond to seasonal changes in the duration of daytime compared to night-time. A plant that has lost photoperiod sensitivity (i.e. it is now photoperiod insensitive) will flower independently of day length while plants sensitive to photoperiod will flower only when days become shorter or longer.

5.2 Relevant biotic factors

42. The major insect pests of sorghum in Queensland are *Helicoverpa armigera* (cotton bollworm) and sorghum midge, which both feed on developing seed (QDAF Insect pest management in sorghum). The applicant may spray insecticide on the GM sorghum to control sorghum midge post-flowering and *Helicoverpa* as required. Agricultural chemicals would be used according to the label instructions, in the same way as for non-GM sorghum.

43. The major disease of sorghum in Australia is ergot, caused by the fungus *Claviceps africana*, which generates alkaloids that are toxic to livestock (GRDC, 2017). Ergot is also toxic to people. Ergot infection occurs during cool and humid weather at flowering, with maximum infection favoured at a constant temperature of 20°C and relative humidity close to 100% (QDAF Disease management for sorghum). The applicant proposes to avoid late planting (i.e. post January) so that flowering occurs during the warmer summer months. If cool weather is forecast during flowering, the applicant may apply a prophylactic fungicide. If there are heavy rainfall events during grain maturity, the applicant may apply fungicide to prevent grain mould.

44. Birds are known to feed on sorghum grain (Mutisya et al., 2016; Xie et al., 2019). Cockatoos and corellas, in particular, are known pests of sorghum in Queensland (ABC Rural news). The proposed Gatton trial site would be enclosed by bird-proof netting. Mice also feed on sorghum, particularly grain, and during plagues have been recorded at populations of up to 3,000 mice/ha in sorghum crops (Kaboodvandpour and Leung, 2008). The applicant proposes to control rodents by baiting.

5.3 Relevant agricultural practices

45. The applicant proposes to plant GM sorghum seed by hand or with precision planters, in rows of 75 cm spacing, with a density of approximately 100,000 plants/ha on irrigated areas within the trial site and 50,000 plants/ha on dryland areas within the trial site. The maximum area planted would be one hectare. The applicant also proposes to bag all sorghum heads to support self-pollination and prevent pollen spread. The bags would be removed after approximately 10 days at the end of flowering, after pollen shed has ceased and pollen grains are no longer viable. If inter-row weeds persist and become unmanageable with manual removal, they may be controlled by spraying with glyphosate or Starane® (fluroxypyr). As mentioned in Section 5.2, spraying with insecticides and fungicides would also take place.

46. Harvesting of sorghum heads would occur by hand or with commercial equipment. If used, all commercial equipment would be cleaned in accordance with the Regulator's requirements. The applicant proposes that the GM sorghum would be threshed and cleaned on site. The trial site would be left fallow during the off-season (May-September) and could be re-planted with the GM sorghum in the following growing season.

5.4 Presence of related plants in the receiving environment

47. As discussed in Section 3, grain sorghum (*Sorghum bicolor* subsp. *bicolor*) is widely cultivated in south-east Queensland. Forage sorghum (*S. bicolor* subsp. *bicolor*), Sudan grass (*S. bicolor* subsp. *drummondii*) or sorghum x Sudan grass hybrids may also be cultivated in the receiving environment (Pacific Seeds summer forage). The wild progenitor of cultivated sorghum, *S. bicolor* subsp. *verticilliflorum* (formerly known as *arundinaceum*), is naturalised in Australia, including south-east Queensland (Atlas of Living Australia). All plants from species *S. bicolor* are diploids that hybridise freely with grain sorghum (OGTR, 2017).

48. Johnson grass (*S. halepense*), Columbus grass (*S. x almum*) and perennial sorghum (*S. spp.* hybrid cv. Silk) are noxious weeds that are naturalised in southern Queensland (National weeds list). These tetraploid species can cross with cultivated sorghum despite ploidy level differences, but hybrid offspring typically have reduced fertility (OGTR, 2017).

49. Wild sorghum relatives are also endemic in many parts of Australia, predominantly in the northern, monsoonal region of the country, mainly occurring in the Northern Territory, Western Australia and Queensland. A recent study reported 17 wild taxa to be native to Australia, with 13 being endemic (Myrans et al., 2020); however, these other *Sorghum* species are not members of the *Eusorghum* section that includes cultivated sorghum. Cultivated sorghum cannot naturally hybridise with species outside *Eusorghum*, due to pollen-pistil incompatibilities and other obstacles (Hodnett et al., 2005).

5.5 Presence of similar genes and encoded proteins in the environment

50. The introduced gene was isolated from a naturally occurring grass species. Further information regarding the presence of similar genes and encoded proteins in the Australian environment has been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

51. Sorghum does possess the genetic potential for asexual seed production without the need for the introduction of new genetic material. For example, through a combination of mutagenesis arising through tissue culture and conventional selection, Belyaeva and colleagues produced an asexual sorghum line that had functional components of apospory (the development of the 2n sexual cell phase, without meiosis and spores), parthenogenesis and autonomous endosperm development (Belyaeva et al., 2021). Thus, genes imparting parthenogenic traits are likely to already be present in sorghum but may not be active due to epigenetic regulation of its DNA (Belyaeva et al., 2021).

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

52. The GM sorghum in this application has not previously been approved for release in Australia.

53. The Regulator has issued a licence for a field trial of other GM sorghum (DIR-153 - Limited and controlled release of sorghum genetically modified for grain quality traits).

54. There have been no approvals for the commercial release of GM sorghum in Australia.

6.2 International approvals

55. The GM sorghum in this application has not previously been approved for release overseas.

56. Different GM sorghum has been approved by other countries. For example, limited field and glasshouse trials of GM sorghum for improved human nutrition (pro-vitamin A, zinc and iron) has been approved by the National Biosafety Authority in Kenya, Burkina Faso and Nigeria (Akinbo et al., 2021).

57. In the United States, the Department of Agriculture has ruled that two types of GM sorghum are not subject to regulation, as they are not plant pests and do not pose an increased noxious weed risk (USDA letter re TRSBG101S transgenic sorghum, USDA letter re TRSBG101B transgenic sorghum).

58. Health Canada has also approved an herbicide tolerant GM sorghum for food use.

Chapter 2 Risk assessment

Section 1 Introduction

59. 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 4). 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.

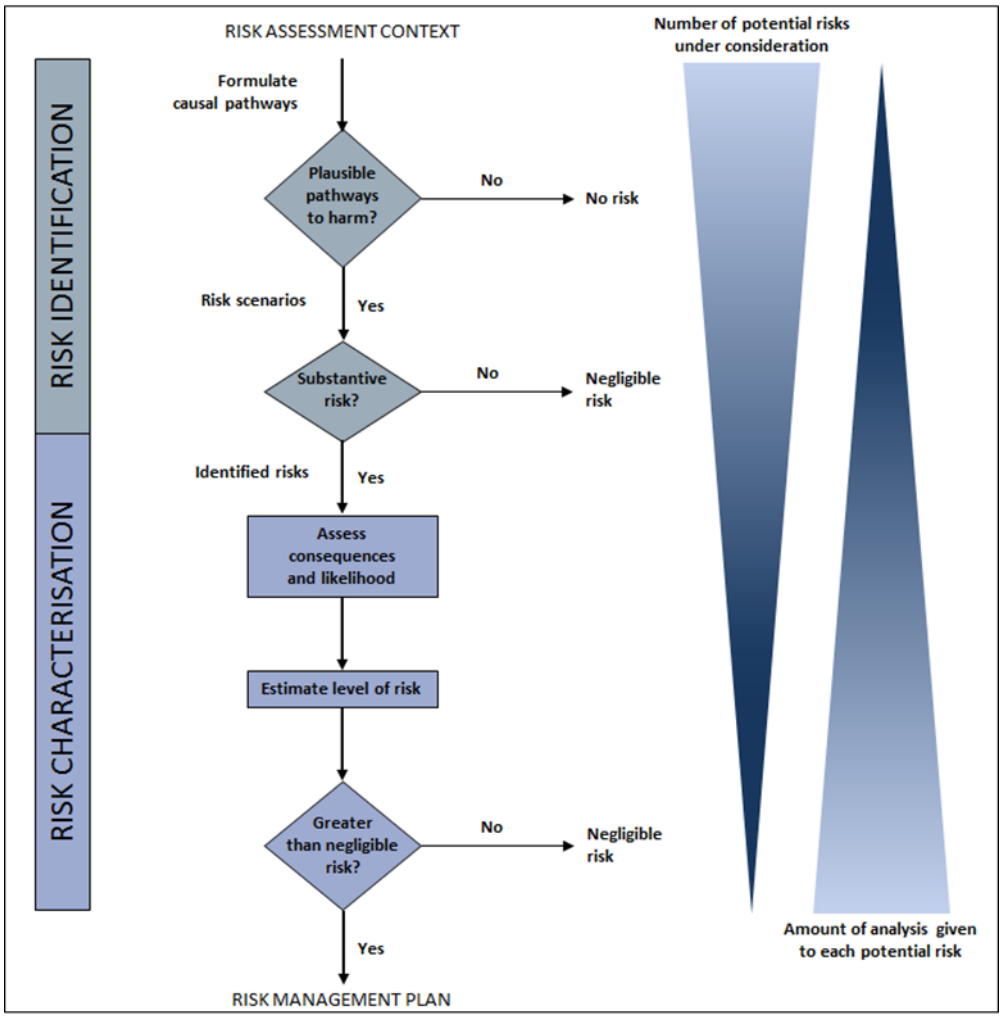

Figure 4. The risk assessment process

60. 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.

61. 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.

62. 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 4), i.e. the risk is considered to be no greater than negligible.

63. Risk scenarios identified as substantive risks are further characterised in terms of the potential seriousness of harm (consequence assessment) and the likelihood of harm (likelihood assessment). The consequence and likelihood assessments are combined 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

64. Postulated risk scenarios are comprised of three components (Figure 5):

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.

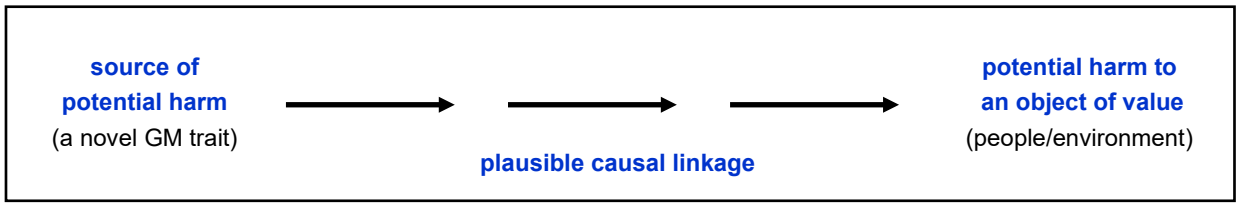

Figure 5. Components of a risk scenario

65. 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

66. 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.

67. As discussed in Chapter 1, the GM sorghum has been modified by the introduction of a gene imparting parthenogenesis. This introduced gene will be considered further as a potential source of risk.

68. During the GM sorghum transformation process, marker genes, and other regulatory elements are introduced to aid in the transformation and selection process. After selection, these regulatory elements would be removed by the Cre-Lox system (reviewed in Scutt et al., 2002). The applicant would undertake molecular testing to confirm that these elements are removed prior to the proposed release of GM sorghum. Therefore, these regulatory elements will not be further considered for this application.

69. The introduced gene is controlled by introduced regulatory sequences derived from plants. Regulatory sequences, such as promoters, enhancer sequences and terminators, 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 are not expressed as proteins, 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.

70. The genetic modifications involving introduction of genes 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. 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). Plants generated by conventional breeding have a long history of safe use, and 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). Therefore, the potential for the processes of genetic modification to result in unintended effects will not be considered further.

2.2 Causal pathway

71. 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 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 GMOs (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. pests, pathogens and weeds)
- tolerance to cultivation management practices
- gene transfer to sexually compatible organisms
- gene transfer by horizontal gene transfer (HGT)
- unauthorised activities.

72. 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.

73. The potential for horizontal gene transfer (HGT) from GMOs, including GM plants, 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: HGT events rarely occur, and the wild-type gene sequences 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.

74. The Act provides for 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, unauthorised activities will not be considered further.

2.3 Potential harm

75. Potential harms from GM plants are based on those used to assess risk from weeds (Virtue et al., 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).

76. 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

77. Five risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 1 and examined in detail in Sections 2.4.1 - 2.4.5 (this Chapter).

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

Table 1. Summary of risk scenarios from the proposed dealings with the GMOs

Risk scenario	Risk source	Causal Pathway	Potential harm	Substantive risk?	Reasons
1	Introduced parthenogenesis gene	Growing GM sorghum at the trial site ⬇ Expression of the parthenogenesis gene in GM sorghum ⬇ Exposure of people who deal with the GM sorghum or of people in the vicinity of the trial site OR Exposure of animals eating GM sorghum	Toxicity or allergenicity to people OR Toxicity to desirable animals	No	• GM sorghum would not be used for human food or animal feed. • The other proposed limits and controls would further restrict exposure of people to the GM sorghum through skin contact or inhalation of pollen; restrict exposure of animals, including birds or desirable insects to the GM sorghum; and exclude large animals from the trial site. • The introduced gene is under the transcriptional control of a tissue-specific promoter and is not expected to be expressed in all tissues in the GM sorghum. • The product of the introduced gene is not expected to be toxic or allergenic.

Risk scenario	Risk source	Causal Pathway	Potential harm	Substantive risk?	Reasons
2	Introduced parthenogenesis gene	Growing GM sorghum at the trial site ⬇ Presence of GM sorghum outside the trial limits ⬇ Establishment of GM sorghum in the environment ⬇ Expression of the parthenogenesis gene in the GM sorghum	Toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment or yield of desirable plants	No	• The proposed limits will restrict the amount of pollen and viable seed available for dispersal. • The proposed controls would minimise persistence of GM sorghum after completion of the trial. • The proposed controls would also minimise dispersal of GM sorghum seed. • Sorghum has limited ability to establish ongoing volunteer populations in the environment. • The parthenogenesis gene cannot be passed down maternally into future generations.
3	Introduced parthenogenesis gene	Growing GM sorghum at the trial site ⬇ Pollen flow to non-GM sorghum or volunteers outside the trial site ⬇ Production of hybrid seed with parthenogenesis gene	Toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment or yield of desirable plants	No	• The proposed limits and controls would minimise pollen flow to non-GM sorghum outside the trial site. • Sorghum has limited ability to establish ongoing volunteer populations in the environment. • The product of the introduced gene is not expected to be toxic or allergenic. • The parthenogenesis gene cannot be passed down maternally into future generations.
4	Introduced parthenogenesis gene	Growing GM sorghum at the trial site ⬇ Outcrossing with sexually compatible weeds ⬇ Introgression of the GM trait into populations of weedy species	Reduced establishment or yield of desirable plants	No	• The proposed limits and controls would minimise outcrossing to sexually compatible weeds. • The parthenogenesis gene cannot be passed down maternally into future generations.

Risk scenario	Risk source	Causal Pathway	Potential harm	Substantive risk?	Reasons
5	Introduced parthenogenesis gene	Growing GM sorghum at the trial site ⬇ Outcrossing to sexually compatible species ⬇ Compatible species possesses a genotype where meiosis is replaced with mitosis OR Spontaneous mutations resulting in meiosis being replaced by mitosis are developed in the cross ⬇ Establishment of asexual plants	Reduced establishment or yield of desirable plants	No	• The proposed limits and controls would minimise outcrossing with compatible species. • Sorghum and many of its compatible species can reproduce by self-pollination. Having an asexual mode of reproduction would still require a single plant to produce offspring. • Asexually reproducing hybrids would have limited genetic flexibility to counteract abiotic and biotic pressures compared to sexually reproducing plants.

2.4.1 Risk scenario 1

Risk source	Introduced parthenogenesis gene
Causal pathway	Growing GM sorghum at the trial site ⬇ Expression of the parthenogenesis gene in GM sorghum ⬇ Exposure of people who deal with the GM sorghum or of people in the vicinity of the trial site OR Exposure of animals eating GM sorghum
Potential harm	Toxicity or allergenicity to people OR Toxicity to desirable animals

Risk source

79. The source of potential harm for this postulated risk scenario is the introduced parthenogenesis gene.

Causal Pathway

80. The parthenogenesis gene is under the transcriptional control of a tissue-specific promoter and is not expected to be expressed throughout the GM sorghum. Further information regarding tissue-specific expression has been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application. Of relevance for this risk assessment is that the gene product would only be expressed in specific tissues, and this would reduce the level of exposure to people and animals during the trial.

Exposure of people to the GM sorghum

81. GM sorghum expressing the introduced parthenogenesis gene would be grown at the trial site. People could be exposed to the GM sorghum through consumption, skin contact or inhalation.

82. The applicant proposes that the GM sorghum will not be used for human food. There is little potential for accidental ingestion of sorghum grown on the trial site. Thus, it is not expected that people would be exposed to the GM sorghum by consumption.

83. The applicant proposes that only trained and authorised staff would be permitted to deal with the GM sorghum. Due to the small scale of the proposed trial, few people would handle the GM sorghum. These people could be exposed to the GM sorghum through skin contact during cultivation, transportation or analysis.

84. As sorghum is wind-pollinated, people could inhale airborne pollen during flowering of the GM sorghum. Pollen shedding from restorer lines (R-lines), such as RTx430 usually lasts for 10-15 days (Singh et al., 1997). The applicant proposes that the GM sorghum flowers would be covered with bags to support self-pollination and limit pollen dispersal. The bags would be removed after pollen shed has ceased and pollen grains are no longer viable (see Section 2.2 of Chapter 1). Bagging GM sorghum flowers would minimise exposure of people to pollen from those plants.

85. If pollen were to escape from the bagged GM sorghum flowers, workers entering the proposed trial site during flowering could be exposed to pollen. A study has reported low levels of sorghum pollen travelling 200 m in the direction of the prevailing wind (Schmidt et al., 2013). Therefore, people in the close vicinity of the proposed trial site during flowering, for instance working in the research stations or nearby farms, could inhale pollen from the GM sorghum. In the event of high winds during flowering, pollen from the GM sorghum could be transported much further than 200 m. However, the severity of allergic reactions to pollen is correlated with atmospheric pollen concentration. If pollen count is below a threshold level (typically around 30 grains/m^3 for grass pollen), this elicits no or minor symptoms even in people sensitive to the pollen allergens (Kiotseridis et al., 2013). Given the small trial site size and the bagging of the flowers, it is not expected that concentrations of airborne pollen from GM sorghum could exceed a threshold level for allergenicity within the trial areas or in areas in close vicinity of the trial sites.

Exposure of animals eating GM sorghum

86. Animals, including birds and desirable insects, entering the trial site could consume GM sorghum. The proposed trial site would be enclosed in bird-proof netting, which would restrict access of large animals and birds to the trial site. The small size and short duration of the proposed trial, combined with the netting, would restrict the numbers of animals that could be exposed to the GM sorghum.

87. The applicant proposes that the GM sorghum would not be used for animal feed. Thus, agricultural livestock would not be exposed to the GM sorghum.

Potential harm

88. 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).

89. The gene product of the introduced gene is not known to be toxic in people or animals, and its orthologs are present in major crops, such as rice and maize (see Chapter 1 Section 4.1). In addition, there is no reasonable expectation that the gene or its product would interact with components in the biochemical pathways for dhurrin production or nitrate accumulation in the GM sorghum. Non-GM sorghum plants naturally produce the toxins dhurrin (which is metabolised to cyanide) and nitrates (which are metabolised to nitrites) (OGTR, 2017). Dhurrin mostly occurs in leaves and is not present in grain, and dhurrin levels are higher in young growth or plants grown under drought conditions

(Doggett, 1988). Nitrates accumulate in stems, leaves and roots rather than flowers or grain, and nitrate levels are higher in young plants or plants grown under unfavourable weather conditions (Sidhu et al., 2011). Sorghum with high levels of dhurrin or nitrates can be toxic to livestock grazing the crop or fed on the hay (Business Queensland - Cyanide and nitrate in sorghum crops). However, neither the introduced gene product nor the GM sorghum have been analysed for toxicity and this remains an area of uncertainty for this risk assessment.

90. No evidence was found in the literature suggesting that non-GM sorghum could be toxic to humans through skin contact or inhalation of pollen, regardless of the levels of native toxins present.

91. Pollen from cultivated non-GM sorghum has been reported to elicit allergic sensitivity in people in India (Davies, 2014). This is also expected to be the case for GM sorghum pollen. Also, a study of grass pollen allergies in Brisbane has identified allergic sensitivity to the pollen of Johnson grass (*S. halepense*) (Davies et al., 2012), which is closely related to cultivated sorghum (*S. bicolor*), so immunological cross-reactivity may exist. There is no expectation that the allergenicity would be increased due to the introduced parthenogenesis gene.

Conclusion

92. Risk scenario 1 is not identified as a substantive risk because the GM sorghum would not be used for human food or animal feed, and other proposed limits and controls would minimise exposure of people and animals to the GM sorghum. In addition, there is no reasonable expectation that expression of the introduced gene could lead to increased toxicity or allergenicity in people or to increased toxicity in animals. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk source	Introduced parthenogenesis gene
Causal pathway	Growing GM sorghum at the trial site ⬇ Presence of GM sorghum outside the trial limits ⬇ Establishment of volunteer GM sorghum in the environment ⬇ Expression of the parthenogenesis gene in the GM sorghum
Potential harm	Toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment or yield of desirable plants

Risk source

93. The source of potential harm for this postulated risk scenario is the introduced parthenogenesis gene.

Causal Pathway

94. GM sorghum would be grown at the trial site and will produce seed. The GM sorghum could also pollinate non-GM sorghum grown as part of the trial, producing seeds.

Presence of the GM sorghum at the trial site after completion of the trial

95. If either live GM sorghum plants or viable seed persisted at the trial site after completion of the trial, this could lead to establishment of volunteer GM sorghum populations in the environment.

96. Grain sorghum is often grown in cultivation as a single-stemmed plant, however, there are tillers at the base of the plants that can develop into additional stems. The extent and timing of tillering depends on both the cultivar and environmental conditions. In some parts of Africa, after harvest of a seeded sorghum crop, a ratoon sorghum crop is grown from the tillers (Doggett, 1988). In Australia, it is a common practice for sorghum growers to desiccate the crop with a knockdown herbicide prior to harvest (GRDC, 2017), which would be expected to prevent further growth of tillers. The applicant has stated they may treat the GM sorghum with glyphosate after it has reached maturity.

97. Some GM sorghum seeds would remain on or in the soil of the trial site after harvesting, for instance, due to seed losses during harvest. These seeds could germinate and grow into volunteer GM sorghum plants. Germination would likely occur soon after the harvest as grain sorghum seed has little dormancy. A study of dormancy in a range of grain sorghum cultivars found that by three months after harvest 93% of seeds germinated, and few of the non-germinated seeds were viable (Gritton and Atkins, 1963). When grain sorghum seeds were buried in soil, < 0.5% of seeds remained viable after four months, and none were viable after eight months (Jacques et al., 1974). There is no reasonable expectation that the introduced parthenogenesis gene would contribute to extending seed dormancy compared to non-GM sorghum.

98. After harvest, the applicant proposes to control persistence of the GM sorghum by mulching and incorporating plants into the soil with the intention to replant at the same site in subsequent seasons until June 2025. The applicant would also undertake post-harvest monitoring every 35 days to identify and destroy any sorghum volunteer plants. This measure is expected to minimise persistence of GM sorghum on the trial site.

Dispersal of GM sorghum outside the trial site

99. Seeds of the GM sorghum could be dispersed outside the trial sites by wind or water, by human activity or by animal activity. Viable seed for dispersal would be present at the trial site after sowing and once the grain has matured.

100. Sorghum seeds are not usually spread by wind as cultivated sorghum has non-shattering seed heads and the seeds lack specialised structures to aid windborne dispersal (OGTR, 2017). It is possible that GM sorghum seeds could be dispersed by high winds if a severe storm occurred while mature seed was present. Sorghum seeds on the soil surface could also be transported by water during heavy runoff or flooding. The applicant reports that the Gatton site is approximately 300 m from the nearest waterway, which would minimise the potential for seed dispersal through flooding.

101. The proposed field trial would occur at a research station, and only people conducting dealings would enter the site. The applicant proposes that all equipment or clothing used in contact with the GMOs would be cleaned before removal from a trial site or use for other purposes. Transport of GM sorghum seeds to and from the trial sites would be conducted in accordance with the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs. These controls would minimise the likelihood of dispersal of GM sorghum seeds from the trial site by people.

102. The applicant has proposed that the GM sorghum would not be used as livestock feed, thus livestock would not be permitted to enter the trial site, and the grain would not be taken off-site for use in feed. Native or exotic animals, such as birds, feral pigs or deer, rabbits, rodents and seed-eating ants could enter the trial site. Some of these may carry seed off the trial site and hoard it; others may eat seed at the trial site and excrete viable seeds in the wider environment.

103. Ants may transport seeds to nest sites approximately two metres on average, but larger distances of over 80 m have also been reported (Gómez and Espadaler, 2013). Mice are likely to

consume sorghum seed on site but they can also collect and carry seed over distances estimated as up to 50 m (Andersson and de Vicente, 2010). The applicant proposes to control rodents in the trial site by baiting. In addition, any seed that is transported a few metres from the parent plant would still be located within areas of the trial site where the applicant proposes to monitor and destroy volunteers (see Chapter 3, Section 3.1.1).

104. Birds, including cockatoos and corellas (ABC Rural news, 2014), and other animals, including feral pigs (The Chronicle, 2017), are known to feed on grain sorghum seed in Queensland. If a proportion of mature sorghum seed can survive digestion without losing viability, this seed could be dispersed in excreta. When pigs eat whole cereal grain, some of the grain passes through undigested and appears as whole grains in manure (Morgan, 2013). Whole sorghum grain may well remain viable after passage through mammalian digestive tracts as germination of sorghum seeds from deer excreta has been reported (Myers et al., 2004). However, chickens efficiently digest whole sorghum seeds, partially because feed does not leave the chicken gizzard until it has been broken down into small particles (Rodgers et al., 2005). Based on the limited evidence available, it seems that sorghum seed dispersal via birds is unlikely, while sorghum seed dispersal via ingestion by mammals is plausible.

105. Furthermore, birds may also hoard or cache seeds. A literature survey has identified only three Australian bird species reported to hoard seeds, all crows or ravens (de Kort and Clayton, 2006) (Queensland Government Department of Environment, accessed 25 May 2022).

106. The applicant proposes that the trial site would be enclosed in bird-proof netting, which would exclude birds and many other animals, including large mammals. This would minimise the potential for seed dispersal by animals.

Ability of the GM sorghum to establish populations in the environment

107. In Australia, volunteer non-GM sorghum plants grow in disturbed sites, such as agricultural areas and roadsides (Groves et al., 2003; Richardson et al., 2011), but volunteer sorghum is not considered a major problem warranting control (Groves et al., 2003). A survey of weed species at farms in the northern grain region of Australia (including south-east Queensland) found that sorghum was the major summer crop grown, providing excellent opportunity for creation of a sorghum seedbank, and that volunteer sorghum plants were present in 54% of paddocks. However, in terms of abundance, volunteer sorghum comprised less than 2% of total weed populations (Rew et al., 2005), indicating that only a small proportion of the sorghum seedbank successfully grew into volunteer plants. This suggests that non-GM cultivated sorghum has limited ability to establish ongoing volunteer populations in the environment.

108. Increased seed production is a factor that contributes to the invasiveness of plants (Keese et al., 2014). Similarly, increased seed size, and the greater resources available to seedlings, can increase a plant's ability to establish amongst competition from existing vegetation, which also contributes to the invasiveness potential of plants (Keese et al., 2014). A study of the grain traits of 65 sorghum cultivars found variation in seed production and seed size (Gambín and Borrás, 2011). Unintentional crossing between the GM sorghum and non-GM sorghum could produce hybrids with both increased seed production and increased seed size. However, these increases are likely to be within the normal range of sorghum cultivars, considering there is no indication that the introduced parthenogenesis gene would have a positive effect on these traits. In fact, the current indication is that, if the introduced gene confers parthenogenesis, half the offspring would be unable to produce seeds at all and a quarter of the offspring would not inherit the introduced gene, meaning it is not present to cause any changes in the traits of the sorghum. Therefore, the GM sorghum and its hybrids, like non-GM sorghum, would have limited ability to establish ongoing volunteer populations in the environment. Furthermore, bagging of the GM flowers would reduce unintentional crossing opportunities.

Potential harm

109. A potential harm from volunteer GM sorghum populations would be toxicity or allergenicity to people. People would not be expected to consume wild sorghum, but they could be exposed to any

GM sorghum through inhalation of pollen. Volunteer GM sorghum could be eaten by desirable animals, including livestock, native animals, including birds, and insect pollinators. Risk scenario 1 discussed the potential for toxicity and allergenicity in people, and toxicity to animals of the GM sorghum and no risk was identified. Furthermore, toxicity from sorghum pollen inhalation has not been observed and the GM sorghum pollen is expected to be as allergenic as the parental non-GM pollen. If volunteer GM sorghum established in commercial non-GM sorghum crops, then people and animals could be exposed through ingestion of food products or feed, respectively. In commercial food or feed production large quantities of seed are pooled and processed. This would mean that GM hybrid seeds would be present at extremely low concentrations in a final food product or feed and would be highly unlikely to elicit toxicity in people or animals.

110. Volunteer GM sorghum plants could compete with and reduce establishment or yield of desirable plants, such as agricultural crops in farms or native plants in nature reserves.

111. If GM sorghum established in a non-GM sorghum crop, its seeds may be reused in subsequent plantings. If the introduced gene imparts parthenogenesis in the female gametes, half of the progeny seeds are expected to grow into haploid and infertile GM sorghum plants (see Chapter 1, Figure 3A). This would decrease the yield of the sorghum crop but would also stop the parthenogenesis gene from being passed down maternally into future generations.

112. Volunteer non-GM sorghum is considered to be a minor problem as a weed in Australian agricultural environments, and a minor and rare problem as a weed in natural environments (Groves et al., 2003). Non-GM sorghum volunteers can be effectively controlled by a range of herbicides (Fleming et al., 2012) as well as physical weed management techniques. The GM sorghum with the introduced parthenogenesis gene is not expected to have increased tolerance to weed management.

Conclusion

113. Risk scenario 2 is not identified as a substantive risk because the proposed limits and controls would minimise dispersal and persistence of GMOs outside the trial, and because sorghum has a limited ability to establish ongoing volunteer populations in the environment. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk scenario 3

Risk source	Introduced parthenogenesis gene
Causal pathway	Growing GM sorghum at the trial site ⬇ Pollen flow to non-GM sorghum or volunteers outside the trial site ⬇ Production of hybrid seed with parthenogenesis gene
Potential harm	Toxicity or allergenicity to people OR Toxicity to desirable animals OR Reduced establishment or yield of desirable plants

Risk source

114. The source of potential harm for this postulated risk scenario is the introduced parthenogenesis gene.

Causal Pathway

115. GM sorghum would be grown at the trial site and would produce pollen. Both the size and the duration of the proposed trial are limited, and, therefore, pollen production would also be limited. If the GM pollen fertilised non-GM cultivated sorghum plants that flowered simultaneously, either crops or volunteers, the non-GM plants would produce hybrid GM seed. The seed could enter animal feed or human food supply chains, be planted or grow into volunteer GM sorghum plants in the environment.

116. Cultivated sorghum is primarily self-pollinating. Outcrossing rates in the field depend on the cultivar and may be up to 30% (Pedersen et al., 1998; Djè et al., 2004). Outcrossing is well known to occur by wind pollination. A South African study reported bees visiting sorghum flowers and pollen adhering to the insects (Schmidt and Bothma, 2005). However, as described in the RARMP for DIR-153, sorghum possesses none of the characteristic features of insect-pollinated plants and all of the characteristic features of wind-pollinated plants and there is no direct evidence of insect-mediated pollination.

117. Two studies of wind-mediated outcrossing in grain sorghum (*S. bicolor* spp. *bicolor*) found that outcrossing between a pollen donor field and pollen recipients occurred at low levels at the maximum distances tested, with one study finding 0.06% outcrossing at 153 m (Schmidt and Bothma, 2006) and the other study finding 0.04% outcrossing at 100 m (Rabbi et al., 2011). It is noted that both studies used male-sterile recipient plants, which were not capable of self-fertilisation, whereas commercial grain sorghum crops are male-fertile, which means that competition from self-pollination would substantially reduce the likelihood of outcrossing. Therefore, outcrossing rates between commercial grain sorghum are expected to be substantially lower than measured in these studies, and immediately neighbouring sorghum would contribute a much higher percentage of the pollen to outcrossing than sorghum which is located at a distance.

118. Another study measured pollen flow from grain sorghum to weedy shattercane (*S. bicolor* spp. *drummondii*) in the direction of the prevailing winds. Shattercane, although self-fertile, has a more open panicle structure than grain sorghum and generally has higher rates of outcrossing. The study found that the average percentage of hybrid seeds produced by the recipient shattercane plants was 0.53% at 100 m and 0.22% at 200 m (Schmidt et al., 2013). Shattercane belongs to the same subspecies and has similar panicle morphology to Sudan grass, and Sudan grass or Sudan grass hybrids are cultivated as forage sorghum in Australia (see Chapter 1, Section 5.4). Therefore, this study provides a model for expected outcrossing rates from grain sorghum to some types of forage sorghum.

119. The applicant has proposed to bag GM sorghum plants during flowering to prevent pollen release, and in addition, the planting area would be surrounded by a 100 m monitoring zone, which would be inspected while the GM sorghum is flowering to destroy any sexually compatible plants. Furthermore, the planting date of GM sorghum would be adjusted to limit synchronous flowering of non-GM sorghum within 300 m of the trial. These proposals would minimise pollen flow from the GM sorghum to either grain or forage sorghum and would minimise the potential for pollen flow to non-GM sorghum outside the trial sites.

120. If GM sorghum pollen fertilised non-GM sorghum, the hybrid seed could be used as animal feed or human food, or for replanting. Alternatively, hybrid seed lost during harvest, spilt during transport or produced by non-GM sorghum volunteer plants could grow as volunteer GM sorghum in the agricultural, intensive use or natural environment. It is to note that half the hybrid seed would be infertile after germination, therefore, the parthenogenesis gene cannot be passed down maternally into future generations (see Chapter 1, Figure 3A).

121. As discussed in Risk scenario 2, the GM sorghum is not expected to have traits, such as increased seed set or increased seed size, which could increase its invasiveness compared to non-GM sorghum. Thus, the GM sorghum would have limited ability to establish ongoing volunteer populations in the environment.

Potential harm

122. If a non-GM sorghum crop produced hybrid GM seeds, it would express the introduced parthenogenesis gene. However, the parthenogenesis gene product is not known to be toxic in people or animals (see Risk scenario 1).

123. If hybrid GM sorghum seeds grew into volunteer plants in the environment, the potential harms would be the same as discussed in Risk scenario 2. Risk scenario 2 was not identified as a substantive risk.

Conclusion

124. Risk scenario 3 is not identified as a substantive risk because the proposed limits and controls would restrict pollen flow to non-GM sorghum outside the trial sites; sorghum has limited ability to establish ongoing volunteer populations in the environment; and the product of the introduced gene is not expected to be toxic or allergenic to people, or toxic to animals. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.4 Risk scenario 4

Risk source	Introduced parthenogenesis gene
Causal pathway	Growing GM sorghum at the trial site ⬇ Outcrossing with sexually compatible weeds ⬇ Introgression of GM trait into populations of weedy species
Potential harm	Reduced establishment or yield of desirable plants

Risk source

125. The source of potential harm for this postulated risk scenario is the introduced parthenogenesis gene.

Causal Pathway

126. GM sorghum would be grown at the trial sites and would produce fertile flowers. Both the size and the duration of the proposed trial are limited, and, therefore, flower production would also be limited. If the GM sorghum outcrossed with weedy related species that flowered simultaneously, this could produce hybrid GM seed. GM hybrid plants could backcross with the weedy parent leading to introgression of GM traits into the weedy species.

127. As described in Chapter 1, Section 5.4, the weedy species that are sexually compatible with grain sorghum and present in south-east Queensland are wild sorghum (*S. bicolor* subsp. *verticilliflorum*; formerly known as *arundinaceum*), Johnson grass, Columbus grass and 'Silk' sorghum. Sorghum crops in Queensland can flower at any time in summer or early autumn, depending on planting dates. Wild sorghum, Johnson grass, Columbus grass and perennial 'Silk' sorghum all have flowering periods that overlap with the potential flowering period of cultivated sorghum (Parsons and Cuthbertson, 2001)(AusGrass2: Sorghum arundinaceum), so could cross with the GM sorghum.

128. Wild sorghum (*S. bicolor* subsp. *verticilliflorum*) belongs to the same species as cultivated sorghum (*S. bicolor* subsp. *bicolor*). The outcrossing potential between cultivated sorghum and wild sorghum is expected to be similar to the outcrossing potentials between two types of cultivated sorghum or between cultivated sorghum and shattercane, which are discussed in Risk scenario 3. A study in Africa found evidence suggesting gene flow from cultivated sorghum to wild sorghum (Sagnard et al., 2011).

129. Despite ploidy differences, hybridisation occurs between grain sorghum and Johnson grass. In a field study, outcrossing between grain sorghum pollen donor fields and Johnson grass plants occurred at an average rate of 1% at 100 m, which was the maximum distance tested (Arriola and Ellstrand, 1996). Some studies of first generation offspring found the hybrids had reduced fertility (reviewed by Warwick and Black, 1983), but other studies found that hybrids had comparable vigour and reproductive ability to the weedy parent (Arriola and Ellstrand, 1997; Magomere et al., 2015). In the United States, a range of alleles originating from cultivated sorghum were found in Johnson grass populations, including weedy populations with no recent exposure to cultivated sorghum, suggesting occurrence of introgression events followed by dispersal (Morrell et al., 2005).

130. Little information is available in the literature about the likelihood of outcrossing, and subsequent introgression of genes, between grain sorghum and Columbus grass or perennial 'Silk' sorghum. It is noted that neither Columbus grass nor perennial 'Silk' sorghum are separate species, but are genetically intermediate between Johnson grass and *S. bicolor* (OGTR, 2017). If outcrossing rates are also intermediate, they would be lower than the outcrossing rate measured for Johnson grass (above) and higher than the outcrossing rate measured for *S. bicolor* (Risk scenario 3).

131. Outcrossing between the GM sorghum and weedy related species could occur either by pollen from the GM sorghum fertilising weeds within or outside the trial site, or by pollen from weeds fertilising the GM sorghum. If pollen from the GM sorghum fertilised a weed, hybrid GM seeds growing on the weed could be widely dispersed. For instance, Johnson grass seeds shatter (Tang et al., 2013) and are transported by wind, water, externally on animals and internally through animal digestive tracts (Parsons and Cuthbertson, 2001). Under normal circumstances, pollen from a weed would not be able to fertilise the egg cell containing the parthenogenesis gene. Fertilisation would only occur in this scenario if the parthenogenesis gene was inactive. Should this occur, the resulting hybrid seeds would have fewer dispersal routes due to the limits and controls proposed in the release. However, the proposed measures to restrict GM seed persistence in the trial sites (Risk scenario 2) might not be sufficient due to high Johnson grass seed dormancy and production of rhizomes that can regrow after an aboveground plant is destroyed (Arriola and Ellstrand, 1997; Magomere et al., 2015).

132. As discussed in Risk scenario 3, the applicant has proposed limits in time and space, and also to bag GM sorghum plants during flowering to prevent pollen release. In addition, the planting area would be surrounded by a 100 m monitoring zone, which would be inspected while the GM sorghum is flowering to destroy any sexually compatible plants. These proposals would minimise pollen flow from the GM sorghum to sexually compatible related weedy species and would also minimise pollination of GM sorghum by sexually compatible related weedy species.

133. As discussed in Chapter 1 Section 4.1.1, should the parthenogenesis gene segregate to the egg cell, the parthenogenesis gene would not be passed on to future generations as the resulting plant would be haploid and not produce seeds. However, depending on the genetic background of the weedy related species, the parthenogenesis gene may be maintained should the weedy relative have mechanisms for asexual seed production (see Risk scenario 5). The parthenogenesis gene may also be maintained should it be introgressed in an inactive state, i.e. silenced due to DNA methylation. Furthermore, should the introduced parthenogenesis gene be introgressed into related weeds, traits related to seed set and seed size, or other traits that contribute to invasiveness, would not be expected to be affected.

Potential harm

134. Non-GM Johnson grass, Columbus grass and perennial 'Silk' sorghum cause a number of harms in the environment and are all declared noxious weeds in NSW and Western Australia, with Johnson grass also declared a weed in NT (National weeds list). Wild sorghum is not a declared noxious weed but is naturalised and known to be a major problem in some agricultural ecosystems in Queensland (Groves et al., 2003). Johnson grass and Columbus grass weeds are difficult to control due to ready regeneration from rhizomes; perennial 'Silk' sorghum has less aggressive rhizomes and is easier to

control (Parsons and Cuthbertson, 2001); wild sorghum has no rhizomes (AusGrass2: Sorghum arundinaceum).

135. Johnson grass pollen has been shown to elicit allergenic sensitivity in people with grass pollen allergies in Brisbane (Davies et al., 2012). The other sorghum weeds are closely related to Johnson grass and may also have allergenic pollen. Johnson grass, Columbus grass, perennial 'Silk' sorghum and wild sorghum are all potentially toxic to livestock, particularly cattle, due to production of dhurrin and nitrates (Parsons and Cuthbertson, 2001; Groves et al., 2003). As discussed in Risk scenario 1, the parthenogenesis gene and its product are not expected to increase allergenicity of the pollen.

136. Risk scenario 1 also discussed that there is no reasonable assumption that the parthenogenesis gene may lead to an increase in toxicity; however, uncertainty was acknowledged. These considerations are also valid should the parthenogenesis gene be introgressed into any of the sexually compatible weedy relatives of sorghum.

137. Johnson grass causes severe crop losses due to direct competition and allelopathic action. Columbus grass is less invasive but can compete with annual crops in high rainfall areas (Parsons and Cuthbertson, 2001). Johnson grass is considered a major problem and Columbus grass and wild sorghum are considered minor problems as weeds in natural ecosystems (Groves et al., 2003). As roadside weeds (an intensive use area), wild sorghum, Johnson grass, Columbus grass and perennial 'Silk' sorghum can all restrict visibility around curves or corners, and all except Johnson grass are also tall enough to obscure signage (Parsons and Cuthbertson, 2001)) (AusGrass2: Sorghum arundinaceum). This is a risk to drivers. The parthenogenesis gene is not known to increase fitness, height or contribute allelopathic interactions. Thus, it is highly unlikely that its presence in any of the sexually compatible weedy relatives would increase the ability of these weed species to reduce the yield or other services in agricultural, natural and intensive use areas.

138. Johnson grass, Columbus grass and perennial 'Silk' sorghum are all known to harbour disease and insect pests that can damage sorghum, maize and sugarcane crops (Parsons and Cuthbertson, 2001). It is assumed that wild sorghum could also be a host for pests and diseases. As the parthenogenesis gene and its product are not known to interact with defence mechanisms in plants, there is no reasonable assumption that its presence would increase the number or range of pests or pathogens hosted on sexually compatible weedy species when compared to the non-GM weedy species.

Conclusion

139. Risk scenario 4 is not identified as a substantive risk because the proposed limits and controls would minimise outcrossing with sexually compatible weeds.

2.4.5 Risk scenario 5

Risk source	Introduced parthenogenesis gene
Causal pathway	Growing GM sorghum at the trial site ⬇ Outcrossing with sexually compatible species ⬇ Compatible species possesses a genotype where meiosis is replaced with mitosis OR Spontaneous mutations resulting in meiosis being replaced by mitosis are developed in the cross ⬇ Establishment of asexual plants
Potential harm	Reduced establishment or yield of desirable plants

Risk source

140. The source of potential harm for this postulated risk scenario is the introduced parthenogenesis gene.

Causal Pathway

141. GM sorghum would be grown at the trial site and would produce pollen within the proposed limits. The GM pollen could fertilise sexually compatible species, including non-GM cultivated sorghum plants (either crops or volunteers) that flowered simultaneously, or weedy species as described in Risk scenario 4.

142. Further information surrounding mode of pollination, outcrossing rates and compatible species have been discussed in Risk scenario 3 and Risk scenario 4.

143. If the recipient plant possessed a genotype where meiosis is replaced by mitosis or spontaneous mutations are developed in the hybrid plant allowing meiosis to be replaced by mitosis, then the GM hybrid plant could reproduce via apomixis, and the resulting seeds would be viable. This could lead to the establishment of populations of GM hybrids that reproduce via apomixis.

144. This change in the reproductive pathway in plants can be brought about by changes to two traits:

- most important would be a knock-out mutation in three genes (*SPO11-1*, *REC8* and *OSD1*), which results in turning meiosis into mitosis (*MiMe*) (see Chapter 1 Section 2). The genes involved in the *MiMe* phenotype are conserved in plants (d'Erfurth et al., 2009) and many grass species contain apomixis traits (Sapkota et al., 2016). It is unknown how likely a spontaneous knock-out mutation in these three genes in the sexually compatible relatives would be; however, unless they are colocalised on a single chromosome this would be highly unlikely.

- A further requirement for asexual seed production is pollen-mediated endosperm fertilisation, which in some apomictic species occurs autonomously (without the need for pollen). Thus pollen, either supplied from the same plant or another plant in the vicinity, would still be required as is the case in non-GM sorghum and its sexually related weedy relatives.

145. In the case of cultivated sorghum, because it is predominantly self-pollinating and requires only one plant to set seed (OGTR, 2017), any GM hybrids reproducing via apomixis would produce similar numbers of seeds as the non-GM sorghum. Weedy related species, such as Johnson grass and Columbus grass can also reproduce via self-pollination (Warwick and Black, 1983) (Tropical Forages). It

is also likely that perennial 'Silk', Sudan grass and wild sorghum (*S. bicolor* subsp. *verticilliflorum*; formerly known as *arundinaceum*) have self-pollination capabilities. Any GM hybrids would therefore produce similar numbers of seed compared to the non-GM parent.

146. Orthologues of the parthenogenesis gene are naturally occurring in the genomes of many grass species including major crops like maize and rice. Thus, the likelihood of these crops outcrossing with their sexually compatible relatives who possess a genotype where meiosis is replaced by mitosis or spontaneous mutations arising in the hybrid plant allowing meiosis to be replaced by mitosis would be similar to that outlined in this risk scenario. However, a key difference in the parthenogenesis gene introduced in the GM sorghum is the tissue-specific promoter controlling the transcriptional regulation of the parthenogenesis gene. The implications of the tissue-specific promoter has been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

147. As discussed in Risk scenario 3, the applicant has proposed to limit the release in time and space, and to bag GM sorghum plants during flowering to prevent pollen release. In addition, the planting area would be surrounded by a 100 m monitoring zone, which would be inspected while the GM sorghum is flowering, and any sexually compatible plants found in this zone would be destroyed. Furthermore, the planting date of GM sorghum would be adjusted to limit synchronous flowering of non-GM sorghum within 300 m of the trial. These proposals would minimise pollen flow from the GM sorghum to non-GM cultivated sorghum plants (either crops or volunteers) and other sexually compatible related weedy species and would also minimise pollination of GM sorghum by sexually compatible related weedy species.

Potential harm

148. The establishment of asexual sorghum or its sexually compatible weedy relatives could give rise to a population that produces more seed (due to a reduction in reproductive barriers) and as a result would have an increased ability to reduce yield or other services in the affected land uses compared to the non-GM sexually compatible relatives.

Conclusion

149. Risk scenario 5 is not identified as a substantive risk because the proposed limits and controls would minimise outcrossing with sexually compatible relatives; and because a number of genetic changes would be necessary to impart functioning apomixis in the GM hybrids. Should establishment of asexual plants occur, existing methods to control weediness would be sufficient.

Section 3 Uncertainty

150. 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.

151. 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.

152. 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.

153. For DIR-189, uncertainty is noted particularly in relation to:

- Potential for increased toxicity or allergenicity of the GM sorghum and
- Potential for the genetic modifications to increase weediness of GM sorghum or hybrids.

154. Overall, the level of uncertainty in this risk assessment is considered low and does not impact on the overall estimate of risk.

155. 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.

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

Section 4 Risk evaluation

157. 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.

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

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

159. Five risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the limits and controls 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 1 and include:

- none of the GM plant material would be used for human food or animal feed
- sorghum has limited ability to establish ongoing volunteer populations in the environment
- limits on the size and duration of the proposed release
- suitability of controls proposed by the applicant to restrict the spread and persistence of the GM sorghum plants and their genetic material.

160. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM sorghum plants into the environment are considered to be 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 additional 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.

Chapter 3 Risk management plan

Section 1 Background

161. 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.

162. 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.

163. 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.

164. 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

165. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed field trial of GM sorghum. These risk scenarios were considered in the context of the scale of the proposed release (Chapter 1, Section 2.1), the proposed controls (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

166. 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 detail in the licence.

3.1 Limits and controls on the release

167. Sections 2.1 and 2.2 in Chapter 1 list the limits and controls proposed by the University of Queensland (UQ). Many of these are discussed in the five risk scenarios considered in Chapter 2. The appropriateness of the limits and controls is considered further in the following sections. Furthermore, many of the control measures replicate licence conditions as issued for DIR-153.

3.1.1 Consideration of limits and controls proposed by UQ

168. The applicant proposes that the duration of the field trial would be limited to three years at a single site with an area of up to 1 ha per year. The small size and short duration of the trial would limit the potential exposure of people and desirable animals to the GMOs (Risk scenario 1).

169. The applicant proposes that only trained and authorised staff would be permitted to deal with the GMOs. Standard licence conditions require all people dealing with the GMOs to be informed of relevant licence conditions. These measures would limit the potential exposure of people to the GMOs (Risk scenario 1).

170. The applicant states that the GM sorghum and its material would not be used for human food or animal feed. Additionally, the GM sorghum has not been assessed for food use by FSANZ. A licence condition prohibits the use of GM plant material in human food or animal feed. This measure would minimise exposure of people or desirable animals to the GM sorghum by consumption (Risk scenario 1).

171. The applicant proposes that any non-GM sorghum plants grown in the trial sites would be treated as if they were GM. This is necessary as the non-GM sorghum plants could be fertilised by GM sorghum pollen and bear GM seed. The applicant also proposes to destroy all GM seed that is not required for analysis or future planting. These practices are imposed as licence conditions and would help minimise persistence or dispersal of GM sorghum seed (Risk scenario 2).

172. At post-harvest, the applicant proposes to control potential persistence of the GM sorghum plants by mulching and incorporating plants into the soil. The applicant also proposes to undertake post-harvest monitoring every 35 days to identify any volunteer plants and then to destroy them. A study found that when grain sorghum seeds were buried in soil, < 0.5% of seeds remained viable after four months, and none were viable after eight months (Jacques et al., 1974). Licence conditions require post-harvest monitoring to continue for at least 12 months and until the trial site has been free of volunteers for at least 6 months, or until the trial site is replanted to the GMOs. This period of monitoring would be appropriate to minimise persistence of GM sorghum seed (Risk scenario 2).

173. In Australia, sorghum crops typically begin to flower 60 days after emergence (GRDC, 2017). However, times from planting to 50% flowering can vary from 55 to 80 days, depending both on cultivar and on temperatures: hot weather hastens flowering and cold weather delays flowering (Spenceley et al., 2005). A study of the parental cultivar of the GM sorghum, RTx430, measured time from planting to 50% flowering as 78 days (Peterson et al., 2009), indicating that it is not an early maturing cultivar. Thus, GM sorghum grown from seed is unlikely to begin flowering earlier than 60 days after emergence except under sustained hot conditions.

174. Sorghum plants can also grow from tillers, and ratoon plants growing from tillers mature more quickly than plants growing from seeds (Doggett, 1988). Therefore, post-harvesting inspections every 35 days would be sufficient to detect emerging sorghum before flowering and this is included as a licence condition.

175. The applicant has not specified which parts of the trial site would be inspected for volunteer sorghum post-harvest. GM sorghum seed lost during harvest and threshing activities could fall a short distance outside the planting areas unobserved, as sorghum seeds are small and inconspicuous. There is also potential for short-distance dispersal of GM sorghum seeds lost during harvest by ants or rodents (Risk scenario 2). Licence conditions require that the planting areas and a 10 m buffer zone surrounding the outer edge of each planting area are subject to post-harvest inspection requirements.

176. The parental cultivar of the GM sorghum, RTx430, germinates poorly at soil temperatures lower than 16°C (Franks et al., 2006) and generally sorghum crops in southern Queensland are planted in October or later to avoid cold conditions. The applicant has proposed control measures as issued in licence DIR-153. Therefore, to promote the germination of volunteers post-harvest, licence conditions require the post-harvest planting areas to be cultivated and irrigated once between October and February.

177. The applicant proposes that field trial sites would be located at least 100 m away from natural waterways, with the Gatton site being reported at least 300 m away. This is intended to manage the

possibility of dispersal of GM sorghum seeds by flooding (Risk scenario 2). Sorghum is, in general, more tolerant to flooding than other cereal crops excluding rice (Hadebe et al., 2017), and a study of flood tolerance of sorghum seed found that on average, over 40% of sorghum seed survived and germinated after 6 days immersion in water (Thseng and Hou, 1993), indicating that long distance dispersal of sorghum seeds by flooding is feasible. Therefore, the proposal that the trial site be located at least 100 m from waterways would minimise the likelihood of seed dispersal by flooding. Another consideration is that sorghum seed could be locally dispersed by high winds or heavy runoff in the event of a severe storm at seed maturity. A standard licence condition requires notification of any extreme weather affecting areas where GMOs may be present to allow assessment and management of any risks.

178. The applicant proposes that all equipment used with the GMOs would be cleaned before use for other purposes or removal from the trial site. The applicant also proposes to transport and store GMOs in accordance with the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs. These controls would restrict the potential for dispersal of GMOs by people (Risk scenario 2).

179. The applicant proposes that rodents in the trial sites would be controlled by baiting. This would restrict the potential for dispersal of GM seed by rodents (Risk scenario 2). Licence conditions require implementation of measures including rodent baits and/or traps to control rodents within the trial sites. Licence conditions also require the innermost 10 m of the monitoring zone to 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. Acceptable measures to achieve this could include keeping land free of vegetation or keeping vegetation mown to a height of less than 10 centimetres.

180. The applicant proposes to enclose the trial site in bird-proof netting, which is expected to exclude birds and larger non-burrowing animals. This would minimise the potential for dispersal of GM seeds from the planting areas by birds or animals as well as the exposure of native animals or birds to the GMOs by consumption (Risk scenario 1 and 2). Therefore, a licence condition to control bird and large animal access requires that the field trial site must be enclosed in a way that is capable of excluding birds, livestock and other large animals.

181. Dispersal of viable seeds by rodents or large animals could occur at planting, while mature seeds are present on the GM plants, or while seeds lost during harvest or threshing are present on the soil surface but have not yet germinated or decomposed. Therefore, licence conditions require control measures for rodents and other animals to be in place from before planting until the planting area is cleaned. Licence conditions define cleaning as destroying sorghum plants, if present, and to remove sorghum seeds from the soil surface to the reasonable satisfaction of the Regulator. The removal of seeds from the soil surface (e.g. tillage) would minimise seed dispersal. Birds are unlikely to feed on sorghum seed at planting as sorghum seeds are typically planted at a depth of 5 cm below the soil surface (GRDC, 2017). Birds would be more likely to feed on sorghum seed before harvest rather than after harvest, as far more seed is present in the field before harvest. Therefore, licence conditions requiring netting to be in place prior to planting the GMOs, while the GMOs are being grown and until the planting area is cleaned would be sufficient.

182. The applicant proposes to manage pollen flow from the GM sorghum by bagging the GM sorghum panicles during flowering and to surround the trial site with a 100 m monitoring zone. Licence conditions require sorghum and related species to be destroyed before flowering or being prevented from flowering simultaneously with the GM sorghum within this zone. Additionally, the applicant proposes to manipulate the planting date of GM sorghum to limit synchronous flowering of cultivated sorghum within 300 m of the trial site. These control measures would minimise outcrossing between the GM sorghum and non-GM sorghum outside the trial sites (Risk scenarios 3, 4 and 5), as well as minimising exposure of people to the GM pollen (Risk scenario 1).

183. When considering pollen flow from the GM sorghum to sexually compatible weeds, one consideration is that weeds would be present at a much lower density than crops, which would reduce the overall outcrossing potential. However, a comparison of outcrossing data at a distance of 100 m suggests a higher outcrossing rate from cultivated sorghum to individual Johnson grass plants than to individual sorghum plants (Arriola and Ellstrand, 1996; Schmidt and Bothma, 2006; Rabbi et al., 2011). Also, the potential harms resulting from outcrossing to weeds (Risk scenarios 4 and 5) may be more serious than the

potential harms resulting from outcrossing to cultivated sorghum (Risk scenario 3). Balancing these factors, it is considered appropriate to separate sexually compatible weeds from the GM sorghum by the same exclusion distance used for non-GM sorghum crops.

184. Therefore, licence conditions require bagging the GM sorghum panicles during flowering and surrounding the trial site with a 100 m monitoring zone. The monitoring zone is required to be inspected while the GM sorghum is flowering and any plants that are sexually compatible with sorghum are required to be destroyed. This condition is expected to minimise outcrossing between the GM sorghum and sexually compatible plants outside the trial sites (Risk scenarios 3, 4 and 5).

3.1.2 Summary of licence conditions to be implemented to limit and control the clinical trial

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

- limit the duration of the release to between September 2022 and June 2025
- limit the size of the release with an area of up to 1 ha
- not allow GM plant material to be used for human food or animal feed
- treat non-GM sorghum grown in the trial sites the same as GM plants
- destroy all GM seed that is not required for analysis or future planting
- monitor the post-harvest trial sites at least every 35 days for a period of at least 12 months, and until the sites are free of sorghum volunteers for at least six consecutive months, and destroy any volunteers found
- cultivate and irrigate the trial site after harvest to encourage germination of seed
- locate the trial sites at least 100 m away from waterways
- clean equipment after use with the GMOs
- transport and store GMOs in accordance with the Regulator's guidelines
- control rodents in the trial sites by baiting and/or trapping
- enclose the trial site in netting, capable of excluding birds and large animals
- control pollen flow by bagging GM sorghum flowers and surrounding the trial site with a 100 m monitoring zone where any sexually compatible plants are destroyed.

3.2 Other risk management considerations

186. 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

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

- any relevant convictions of the applicant
- 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.

188. On the basis of information submitted by the applicant and records held by the OGTR, the Regulator considers the University of Queensland suitable to hold a licence. The licence includes a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

189. In addition, the applicant organisation must have access to an IBC and be an accredited organisation under the Act.

3.2.2 Contingency plans

190. As per licence conditions, UQ is 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 sorghum outside permitted areas.

191. Before planting the GMOs, UQ is also 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

192. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to growing the GMOs, UQ is required to provide a list of people and organisations that would be covered by the licence, or the function or position where names are not known at the time.

3.2.4 Reporting requirements

193. The licence requires 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 dealings
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the field trial.

194. A number of written notices are also required under the licence regarding dealings with the GMO, to assist the Regulator in designing and implementing a monitoring program for all licensed dealings. The notices 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

195. 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 would continue until the Regulator is satisfied that all the GMOs resulting from the authorised dealings have been removed from the release sites.

196. 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.

197. 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 the health and safety of people or the environment could result.

Section 4 Issues to be addressed for future releases

198. Additional information has been identified that may be required to assess an application for a commercial release of the GM sorghum or to justify a reduction in limits and controls. This includes:

- additional molecular and biochemical characterisation of the GM sorghum plants, particularly with respect to potential for increased toxicity or allergenicity
- additional phenotypic characterisation of the GM sorghum, particularly with respect to potential for increased weediness
- additional data regarding effects that may increase the weediness of a weedy relative after introgression of the parthenogenesis gene.

Section 5 Conclusions of the RARMP

199. The risk assessment concludes that the proposed limited and controlled release of GM sorghum poses negligible risks to the health and safety of people or the environment as a result of gene technology. These negligible risks do not require specific risk treatment measures.

200. Licence conditions are 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.

References

ABARES (2021). Agricultural commodities and trade data - Crop data underpinning: Australian crop report: December 2021 No. 200. (Australian Bureau of Agricultural and Resource Economics and Sciences; available online).

Akinbo, O., Obukosia, S., Ouedraogo, J., Sinebo, W., Savadogo, M., Timpo, S., Mbabazi, R.*, et al.* (2021). Commercial release of genetically modified crops in Africa: Interface between biosafety regulatory systems and varietal release systems. Frontiers in Plant Science *12*.

Andersson, M.S., and de Vicente, M.C. (2010). Sorghum. In Gene Flow between Crops and Their Wild Relatives (The Johns Hopkins University Press), pp. 443-462.

Arriola, P.E., and Ellstrand, N.C. (1996). Crop-to-weed gene flow in the genus *Sorghum* (Poaceae): Spontaneous interspecific hybridization between Johnsongrass, *Sorghum halepense*, and crop sorghum, *S. bicolor.* American Journal of Botany *83*, 1153-1160.

Arriola, P.E., and Ellstrand, N.C. (1997). Fitness of interspecific hybrids in the genus *Sorghum*: Persistence of crop genes in wild populations. Ecological Applications *7*, 512-518.

Arts, J.H.E., Mommers, C., and de Heer, C. (2006). Dose-response relationships and threshold levels in skin and respiratory allergy. Critical Reviews in Toxicology *36*, 219-251.

Belyaeva, E.V., Elkonin, L.A., Vladimirova, A.A., and Panin, V.M. (2021). Manifestation of apomictic potentials in the line AS-3 of *Sorghum bicolor* (L.) Moench. Planta *254*, 37.

Cuevas, H.E., Zhou, C., Tang, H., Khadke, P.P., Das, S., Lin, Y.R., Ge, Z.*, et al.* (2016). The evolution of photoperiod-insensitive flowering in sorghum, a genomic model for panicoid grasses. Molecular Biology and Evolution *33*, 2417-2428.

Cummings, J.L., Handley, L.W., MacBryde, B., Tupper, S.K., Werner, S.J., and Byram, Z.J. (2008). Dispersal of viable row-crop seeds of commercial agriculture by farmland birds: implication for genetically modified crops. Environmental Biosafety Research *7*, 241-252.

d'Erfurth, I., Jolivet, S., Froger, N., Catrice, O., Novatchkova, M., and Mercier, R. (2009). Turning Meiosis into Mitosis. PLOS Biology *7*, e1000124.

Davies, J.M. (2014). Grass pollen allergens globally: the contribution of subtropical grasses to burden of allergic respiratory diseases. Clinical & Experimental Allergy *44*, 790-801.

Davies, J.M., Li, H., Green, M., Towers, M., and Upham, J.W. (2012). Subtropical grass pollen allergens are important for allergic respiratory diseases in subtropical regions. Clinical and Translational Allergy *2*, 4.

de Kort, S.R., and Clayton, N.S. (2006). An evolutionary perspective on caching by corvids. Proceedings of the Royal Society B: Biological Sciences *273*, 417-423.

Djè, Y., Heuertz, M., Ater, M., Lefèbvre, C., and Vekemans, X. (2004). In situ estimation of outcrossing rate in sorghum landraces using microsatellite markers. Euphytica *138*, 205-212.

Doggett, H. (1988). Sorghum, 2nd edn (Essex, UK: Longman Scientific and Technical).

Felsot, A.S. (2000). Insecticidal genes part 2: Human health hoopla. Agrichemical & Environmental News *168*, 1-7.

Fleming, J., McNee, T., Cook, T., and Manning, B. (2012). Weed control in summer crops 2012-13. (NSW Department of Primary Industries; available online).

Franks, C.D., Burow, G.B., and Burke, J.J. (2006). A comparison of U.S. and Chinese sorghum germplasm for early season cold tolerance. Crop Science *46*, 1371-1376.

Gambín, B.L., and Borrás, L. (2011). Genotypic diversity in sorghum inbred lines for grain-filling patterns and other related agronomic traits. Crop & Pasture Science *62*, 1026-1036.

Gómez, C., and Espadaler, X. (2013). An update of the world survey of myrmecochorous dispersal distances. Ecography *36*, 1193-1201.

Grain Growers (2021). State of the Australian grains industry 2021. (Grain Growers Limited; available online).

GRDC (2017). GRDC Grownotes: Sorghum. (Canberra: Grains Research and Development Corporation; availaible online).

Gritton, E.T., and Atkins, R.E. (1963). Germination of sorghum seed as affected by dormancy. Agronomy Journal *55*, 169-174.

Groves, R.H., Hosking, J.R., Batianoff, G.N., Cooke, D.A., Cowie, I.D., Johnson, R.W., Keighery, G.J.*, et al.* (2003). Weed categories for natural and agricultural ecosystem management (Bureau of Rural Sciences, Canberra).

Hadebe, S.T., Modi, A.T., and Mabhaudhi, T. (2017). Drought tolerance and water use of cereal crops: A focus on sorghum as a food security crop in sub-Saharan Africa. Journal of Agronomy and Crop Science *203*, 177-191.

Higgins, R.H., Thurber, C.S., Assaranurak, I., and Brown, P.J. (2014). Multiparental mapping of plant height and flowering time QTL in partially isogenic sorghum families. G3 Genes|Genomes|Genetics *4*, 1593-1602.

Hodnett, G.L., Burson, B.L., Rooney, W.L., Dillon, S.L., and Price, H.J. (2005). Pollen-pistil interactions result in reproductive isolation between *Sorghum bicolor* and divergent *Sorghum* species. Crop Science *45*, 1403-1409.

Jacques, G.L., Vesecky, J.F., Feltner, K.C., and Vanderlip, R.L. (1974). Effects of depth and duration of burial on shattercane seed. Crop Science *14*, 787-789.

Kaboodvandpour, S., and Leung, L.K.-P. (2008). Does the house mouse self-regulate its density in maturing sorghum and wheat crops? Journal of Animal Ecology *77*, 1030-1037.

Keese, P. (2008). Risks from GMOs due to horizontal gene transfer. Environmental Biosafety Research *7*, 123-149.

Keese, P.K., Robold, A.V., Myers, R.C., Weisman, S., and Smith, J. (2014). Applying a weed risk assessment approach to GM crops. Transgenic Research *23*, 957-969.

Kiotseridis, H., Cilio, C.M., Bjermer, L., Tunsäter, A., Jacobsson, H., and Dahl, Å. (2013). Grass pollen allergy in children and adolescents-symptoms, health related quality of life and the value of pollen prognosis. Clinical and Translational Allergy *3*, 19.

Magomere, T.O., Obukosia, S.D., Shibairo, S.I., Ngugi, E.K., and Mutitu, E. (2015). Evaluation of relative competitive ability and fitness of *Sorghum bicolor*×*Sorghum halepense* and *Sorghum bicolor*×*Sorghum sudanense* F1 hybrids. Journal of Biological Sciences *15*, 1-15.

Morgan, J. (2013). A diet fit for a pig: seven basic rules. (NSW Department of Primary Industries; available online).

Morrell, P.L., Williams-Coplin, T.D., Lattu, A.L., Bowers, J.E., Chandler, J.M., and Paterson, A.H. (2005). Crop-to-weed introgression has impacted allelic composition of johnsongrass populations with and without recent exposure to cultivated sorghum. Molecular Ecology *14*, 2143-2154.

Mutisya, D.L., Karanja, D.R., and Kisilu, R.K. (2016). Economic advantage of sorghum harvest at soft dough grain stage to prevent bird damage. Cogent Food & Agriculture *2*, 1259141.

Myers, J.A., Vellend, M., Gardescu, S., and Marks, P.L. (2004). Seed dispersal by white-tailed deer: implications for long-distance dispersal, invasion, and migration of plants in eastern North America. Oecologia *139*, 35-44.

Myrans, H., Diaz, M.V., Khoury, C.K., Carver, D., Henry, R.J., and Gleadow, R. (2020). Modelled distributions and conservation priorities of wild sorghums (*Sorghum* Moench). Diversity and Distributions *26*, 1727-1740.

Nguyen, C.T., Singh, V., van Oosterom, E.J., Chapman, S.C., Jordan, D.R., and Hammer, G.L. (2013). Genetic variability in high temperature effects on seed-set in sorghum. Functional Plant Biology *40*, 439-448.

OGTR (2013). Risk Analysis Framework 2013, 4th edn (Canberra: Office of the Gene Technology Regulator, available online).

OGTR (2017). The Biology of *Sorghum bicolor* (L.) Moench subsp. *bicolor* (Sorghum) Report No. Version 1.1. (available online: Office of the Gene Technology Regulator).

Parsons, W.T., and Cuthbertson, E.G. (2001). Noxious Weeds of Australia, 2nd edn (Collingwood, Victoria: CSIRO Publishing).

Pedersen, J.F., Toy, J.J., and Johnson, B. (1998). Natural outcrossing of sorghum and sudangrass in the central grass plains. Crop Science *38*, 937-939.

Peterson, G.C., Schaefer, K., and Pendleton, B.B. (2009). Registration of 16 sorghum germplasm lines. Journal of Plant Registrations *3*, 203-205.

Philp, T., Peake, A., and McLean, G. (2010). Improving profitability of irrigated sorghum through partial irrigation of larger areas. Paper presented at: Proceedings of the 1st Australian Summer Grains Conference (Gold Coast, Australia).

Rabbi, I.Y., Parzies, H.K., Kiambi, D., Haussmann, B.I., Folkertsma, R., and Geiger, H.H. (2011). Experimental studies on pollen-mediated gene flow in *Sorghum bicolor* (L.) Moench using male-sterile bait plants. Plant Breeding *130*, 217-224.

Rew, L.J., Medd, R.W., Van de Ven, R., Gavin, J.J., Robinson, G.R., Tuitee, M., Barnes, J.*, et al.* (2005). Weed species richness, density and relative abundance on farms in the subtropical grain region of Australia. Australian Journal of Experimental Agriculture *45*, 711-723.

Richardson, F.J., Richardson, R.G., and Shepherd, R.C.H. (2011). Weeds of the South-East: an identification guide for Australia, 2nd edn (Meredith, Victoria, Australia: R.G. and F.J. Richardson).

Rodgers, N., Choct, M., Hetland, H., Sundby, F., and Svihus, B. (2005). Dietary roller-milled sorghum improves broiler efficiency and whole sorghum improves gut development. Recent Advances in Animal Nutrition in Australia *15*, 57-64.

Sagnard, F., Deu, M., Dembele, D., Leblois, R., Toure, L., Diakite, M., Calatayud, C., *et al.* (2011). Genetic diversity, structure, gene flow and evolutionary relationships within the *Sorghum bicolor* wild-weedy-crop complex in a western African region. Theoretical and Applied Genetics *123*, 1231-1246.

Sapkota, S., Conner, J.A., Hanna, W.W., Simon, B., Fengler, K., Deschamps, S., Cigan, M., *et al.* (2016). *In silico* and fluorescence *in situ* hybridization mapping reveals collinearity between the *Pennisetum squamulatum* apomixis carrier-chromosome and chromosome 2 of sorghum and foxtail millet. PLOS ONE *11*, e0152411.

Schmidt, J.J., Pedersen, J.F., Bernards, M.L., and Lindquist, J.L. (2013). Rate of shattercane x sorghum hybridization in situ. Crop Science *53*, 1677-1685.

Schmidt, M., and Bothma, G. (2006). Risk assessment for transgenic sorghum in Africa: Crop-to-crop gene flow in *Sorghum bicolor* (L.) Moench. Crop Science *46*, 790-798.

Schmidt, M.R., and Bothma, G. (2005). Indications of bee pollination in sorghum and its implications for transgenic biosafety. International Sorghum and Millets Newsletter *6*, 72-75.

Schnell, J., Steele, M., Bean, J., Neuspiel, M., Girard, C., Dormann, N., Pearson, C., *et al.* (2015). A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments. Transgenic Research *24*, 1-17.

Scutt, Charles P., Zubko, E., and Meyer, P. (2002). Techniques for the removal of marker genes from transgenic plants. Biochimie *84*, 1119-1126.

Sidhu, P.K., Bedi, G.K., Meenakshi, Mahajan, V., Sharma, S., Sandhu, K.S., and Gupta, M.P. (2011). Evaluation of factors contributing to excessive nitrate accumulation in fodder crops leading to ill-health in dairy animals. Toxicology International *18*, 22-26.

Singh, F., Rai, K.N., Reddy, B.V.S., and Diwakar, B. (1997). Development of cultivars and seed production techniques in sorghum and pearl millet. (India: ICRISAT).

Society of Toxicology (2003). Society of Toxicology position paper: The safety of genetically modified foods produced through biotechnology. Toxicological Sciences *71*, 2-8.

Spenceley, J., Butler, G., Nicholas, A., Simpfendorfer, S., Holland, J., Kniepp, J., Edwards, J., *et al.* (2005). Grain Sorghum. (NSW Department of Primary Industries; available online).

Steiner, H.Y., Halpin, C., Jez, J.M., Kough, J., Parrott, W., Underhill, L., Weber, N., *et al.* (2013). Evaluating the potential for adverse interactions within genetically engineered breeding stacks. Plant Physiology *161*, 1587-1594.

Tang, H., Cuevas, H.E., Das, S., Sezen, U.U., Zhou, C., Guo, H., Goff, V.H., *et al.* (2013). Seed shattering in a wild sorghum is conferred by a locus unrelated to domestication. Proceedings of the National Academy of Sciences *110*, 15824-15829.

Thseng, F., and Hou, F. (1993). Varietal differences and diallel analysis of pre-germination flooding tolerance of sorghum seed. Japanese Journal of Breeding *43*, 23-28.

Vijverberg, K., Ozias-Akins, P., and Schranz, M.E. (2019). Identifying and engineering genes for parthenogenesis in plants. Frontiers in Plant Science *10*.

Virtue, J.G., Spencer, J.E., Weiss, J.E., and Reichard, S.E. (2008). Australia's Botanic Gardens weed risk assessment procedure. Plant Protection Quarterly *23*, 166-178.

Warwick, S.I., and Black, L.D. (1983). The biology of Canadian weeds. 61 *Sorghum halepense* (L.) PERS. Journal of Plant Science *63*, 997-1014.

Xie, P., Shi, J., Tang, S., Chen, C., Khan, A., Zhang, F., Xiong, Y.*, et al.* (2019). Control of bird feeding behavior by *Tannin1* through modulating the biosynthesis of polyphenols and fatty acid-derived volatiles in sorghum. Molecular Plant *12*, 1315-1324.

Appendix A: Summary of submissions from prescribed experts, agencies and authorities on the consultation RARMP

The Regulator received several submissions from prescribed experts, agencies and authorities[5] on the consultation RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence and were used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence. Advice received is summarised below.

Submission	Summary of issues raised	Comment
1	Agrees with the overall conclusion of the RARMP. Agrees that all plausible risk scenarios have been identified and did not identify additional relevant information that should be considered. Agrees that the proposed limits and controls for the GM sorghum are appropriate.	Noted.
2	Notes that the licence will prohibit the use of GM plant material in human food or animal feed. Does not have any further comments on the licence application at this stage.	Noted.
3	Satisfied with the conclusions of the RARMP and has no comments.	Noted.
4	Has concerns about the Gatton field trial site and the glasshouses at the University of Queensland St. Lucia campus flooding and that flood related escapes of the GM sorghum have not been adequately addressed.	Risk scenario 2 in Chapter 2 discusses the risks from dispersal of the GM sorghum, including the likelihood of dispersal by flooding. The licence for DIR 189 authorises dealings with the GM sorghum at the Gatton field trial site, but not at the St Lucia campus. The proposed Planting Area was not flooded during the recent and previous major flooding events. A range of licence conditions manage risks from potential flooding of the field trial site, including requiring and enacting contingency plans as necessary.
5	Have no concerns with this application.	Noted.
6	Accepts that, overall, the University of Queensland's application has negligible risks to the health and safety of people and the environment. Satisfied that the measures taken to manage the short- and long-term risks from the proposal are adequate.	Noted.

[5] Prescribed expects, agencies and authorities include GTTAC, State and Territory Governments, Australian Government agencies and the Minister for the Environment.

Appendix B: Summary of submissions from the public on the consultation RARMP

The Regulator received one submission from a member of the public on the notification of the application and one submission from the public on the consultation RARMP. The issues raised in the submission are summarised in the table below. All issues that related to risks to the health and safety of people and the environment were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Submission	Summary of issues raised	Comment
1	Is pleased to note the development of this type of asexual GM plant for use in Australia and that it would not be used for human food production. Notes that a foreseeable consequence of production of a GM crop with fertile seed would be the wind dispersal leading to germination of volunteer plants in neighbouring fields and properties.	Noted.
2	"Is there no end to this GM madness?"	Noted.