Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 158

Commercial release of safflower genetically modified for high oleic acid composition

Applicant: GO Resources Pty Ltd

June 2018

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application DIR 158

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application for the intentional, commercial scale release of genetically modified (GM) safflower in Australia. A Risk Assessment and Risk Management Plan (RARMP) for this application was prepared by the Regulator in accordance with the requirements of 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 concludes that this commercial release poses negligible risks to human health and safety and the environment and no specific risk treatment measures are imposed. However, general licence conditions have been imposed to ensure that there is ongoing oversight of the release.

The application

Application number	DIR 158
Applicant	GO Resources Pty Ltd (GO Resources)
Project title	Commercial release of safflower genetically modified for high oleic acid composition[1]
Parent organism	*Carthamus tinctorius* L. (safflower)
Introduced gene and modified trait	**Two gene fragments involved in altered fatty acid composition:** • Fragment of *CtFATB* (palmitoyl-ACP-thioesterase), derived from safflower • Fragment of *CtFAD2.2* (Δ12 desaturase), derived from safflower **One selectable marker gene:** • *Hph* (hygromycin phosphotransferase), from *Streptomyces* sp., antibiotic resistance gene
Proposed locations	Australia-wide
Primary purpose	Commercial release of the GM safflower

Risk assessment

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

The risk assessment process considers how the genetic modification and activities conducted with the GMO 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 information in the application, relevant previous

[1] The title of the application submitted by GO Resources is "Commercial release of *Carthamus tinctorius* L. genetically modified for high oleic acid composition".

approvals, current scientific knowledge and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP. Both the short and long term risks are considered.

Credible pathways to potential harm that were considered included: toxic and allergenic properties of the GM safflower; potential for increased weediness of the GM safflower relative to unmodified plants; and vertical transfer of the introduced genetic material to other sexually compatible plants.

The principal reasons for the conclusion of negligible risks are: the introduced genetic modifications are not considered to produce compounds that are toxic or allergenic to people or toxic to other desirable organisms; genes similar to the introduced gene constructs are widespread in the environment; the GM safflower was licenced for field trials in Australia from 2013, with no reported adverse or unexpected effects; and the GM safflower has limited capacity to survive in natural habitats.

Risk management

The risk management plan concludes that risks from the proposed dealings can be managed so as to protect people and the environment by imposing general conditions to ensure that there is ongoing oversight of the release.

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 evaluates and treats identified risks and considers general risk management measures. The risk management plan is given effect through licence conditions.

As the level of risk is assessed as negligible, specific risk treatment is not required. However, the Regulator has imposed licence conditions regarding post-release review (PRR) to ensure that there is ongoing oversight of the release and to allow the collection of information to verify the findings of the RARMP. The licence also contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements, which include an obligation to report any unintended effects.

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Abbreviations

ASTAG	Australian Strategic and Technical Advisory Group on Antimicrobial Resistance
APVMA	Australian Pesticides and Veterinary Medicines Authority
cm	Centimetre(s)
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
FA	Fatty acid
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GMO	Genetically modified organism
ha	hectare
HGT	Horizontal gene transfer
HPT	Hygromycin B phosphotransferase
km	Kilometre(s)
LOR	Limit of reporting
m	Metre(s)
mg	Milligram(s)
mL	Millilitre(s)
NHMRC	National Health and Medical Research Council
ng	Nanogram(s)
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
OIE	World Organisation for Animal Health
ORF	Open reading frame
ppm	Parts per million
PRR	Post release review
RARMP	Risk Assessment and Risk Management Plan
RCBD	Randomised complete block design
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
RMP	Resistance management plan
spp.	Species
TGA	Therapeutic Goods Administration
the Act	The *Gene Technology Act 2000*
WHO	World Health Organization

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 in conjunction with the Gene Technology Regulations 2001 (the Regulations), an inter-governmental agreement and corresponding legislation in States and Territories, 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. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

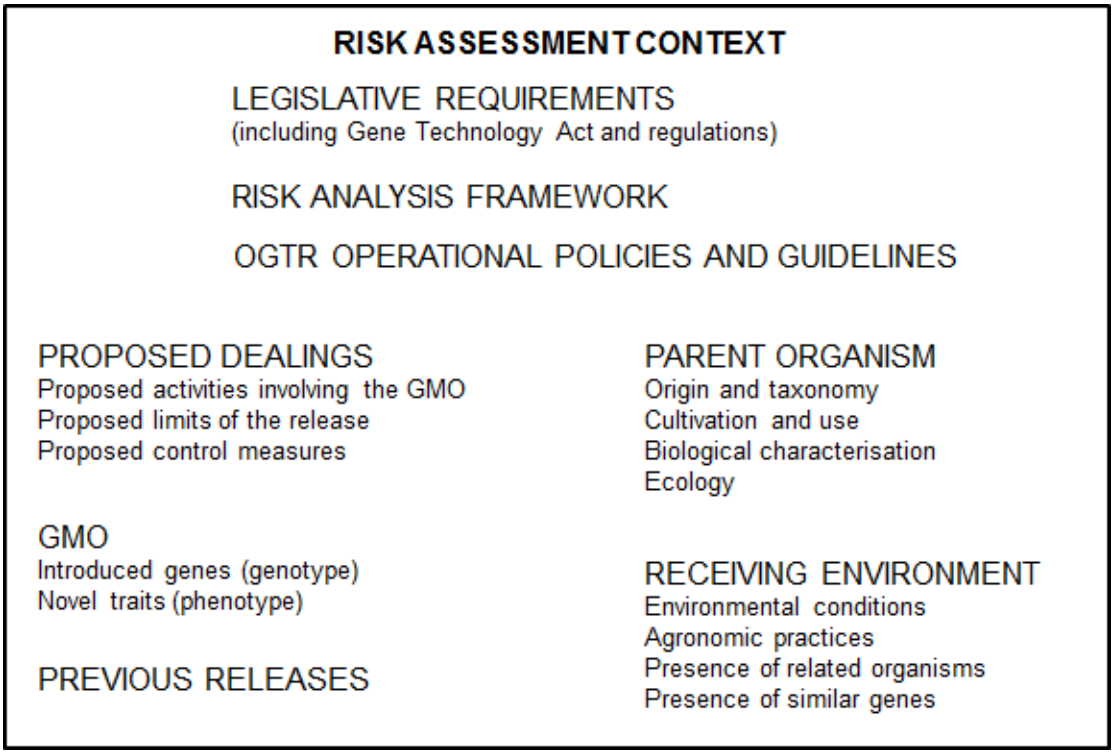

Figure 1 Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and who must be consulted, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that inform the decisions on licence applications. In addition, the Regulations outline further matters the Regulator must consider when preparing a RARMP.

5. Since this application is for commercial purposes, it cannot be considered as a limited and controlled release application under section 50A of the Act. Therefore, under section 50(3) of the Act, the Regulator was required to seek advice from prescribed experts, agencies and authorities on matters

relevant to the preparation of the RARMP. This first round of consultation included the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, all Australian local councils[2] and the Minister for the Environment. A summary of issues contained in submissions received is given in Appendix A.

6. Section 52 of the Act requires the Regulator, in a second round of consultation, to seek comment on the RARMP from the experts, agencies and authorities outlined above, as well as the public. Advice from the prescribed experts, agencies and authorities in the second round of consultation, and how it was taken into account, is summarised in Appendix B. One public submission was received and its consideration is summarised in Appendix C.

7. The Risk Analysis Framework (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the legislative requirements. Additionally, there are a number of operational policies and guidelines developed by the Office of the Gene Technology Regulator (OGTR) that are relevant to DIR licences. These documents are available from the OGTR website.

8. Any dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration and the Department of Agriculture and Water Resources. These dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

Section 3 The proposed release

9. GO Resources Pty Ltd (GO Resources) proposes commercial cultivation of two genetically modified (GM) safflower lines, Event 26 and Event 40. Each line contains an RNAi gene silencing construct that targets two endogenous safflower fatty acid biosynthesis genes, involved in the conversion of oleic acid to linoleic acid or palmitic acid. These two events are known by their unique OECD identifiers, GOR-73226-6 (Event 26) and GOR-7324Ø-2 (Event 40), respectively.

10. The applicant is seeking approval for the release to occur Australia-wide, subject to any moratoria imposed by States and Territories for marketing purposes. The GM safflower could be grown in all commercial safflower growing areas, and products derived from the GM plants would enter general commerce. The applicant intends that the oil derived from the GM safflower will be used for commercial industrial oil production and the meal derived from the GM safflower will be used as stock feed.

11. The dealings involved in the proposed intentional release are to:

(a) conduct experiments with the GMO

(b) breed the GMO

(c) propagate the GMO

(d) use the GMO in the course of manufacture of a thing that is not a GMO

(e) grow the GMO

(f) import the GMO

[2] GO Resources is seeking approval for unrestricted commercial release of the GM safflower lines in all safflower growing areas of Australia. Safflower may be grown over a significant proportion of Australian agricultural land, and viable safflower seed may be transported out of the safflower growing areas. Therefore, the Regulator decided to consult with all of the local councils in Australia, except for those that have requested not to be consulted on such matters.

(g) transport the GMO

(h) dispose of the GMO

and the possession, supply or use of the GMO for the purposes of, or in the course of, any of the above.

Section 4 The parent organism

12. The parent organism of the GMOs is safflower (*Carthamus tinctorius* L.), a member of the daisy plant family (Asteraceae). In establishing the risk context, details of the parent organism form part of the baseline for a comparative risk assessment (Figure 1, OGTR, 2013). Non-GM safflower is the standard baseline for biological comparison. Detailed information about the parent organism is contained in the reference document *The Biology of* Carthamus tinctorius L. *(safflower)* (OGTR, 2018), which was produced to inform the risk assessment process for licence applications involving GM safflower. This document is available from the OGTR website. Some of the information from this document is summarised below.

13. Safflower is exotic to Australia and is cultivated as an annual oilseed crop. It is a branching thistle-like herbaceous plant with spiny leaves (Singh and Nimbkar, 2006). Safflower is generally planted in the winter or early spring in Australia. Safflower is fairly slow-growing with a period of 18-31 weeks between sowing and maturity, depending on cultivar, sowing time and weather conditions (GRDC, 2010).

14. Safflower is either self-pollinated or insect pollinated and its pollen is not transported appreciably by wind beyond 1 m (Claassen, 1950). Many safflower varieties are 85-90% self-pollinating with insects, primarily honey bees, responsible for the remaining 10-15% (USDA-APHIS, 2008). Outcrossing rates between safflower plants in close proximity (1-1.5 m) appear to be highly variable and can range from 0-100% (Claassen, 1950) with an average outcrossing rate of 10% (GRDC, 2010). Long distance outcrossing between safflower plants has been reported in a single experiment to occur at a rate of 0.12% at 50 m and 0.01% at 100 m (McPherson et al., 2009a). Industry standards suggest an isolation distance of 400 m for producing basic safflower seed, which is planted to produce certified seed (OECD, 2013).

15. Safflower reproduces by seeds, which are smooth and fairly large, 6-7 mm, each weighing approximately 40 mg (GRDC, 2010). The seed heads are highly resistant to shattering. Safflower seeds have very low dormancy and ripe seeds may germinate in the head following rainfall. The little seed dormancy reported in safflower is lost during storage. Viable safflower seed persistence in the seed bank is less than two years at the soil surface and less than one year if the seeds are buried in the soil (McPherson et al., 2009b). Animal predation of safflower is limited due to its spiny nature. Bird predation of safflower seed occurs, but studies of some bird species (blackbirds, mallard ducks, pheasants and pigeons) show that seeds that have passed through the digestive systems are no longer viable (Cummings et al., 2008; GRDC, 2010).

16. Safflower seed oil and the seed meal are generally not considered to be toxic and have a long history of safe use. However, anti-nutrient compounds such as lignan glucosides and tannins and natural toxins such as hydrogen cyanide and oxalates are present in the seed (Ingale and Shrivastava, 2011; Kuehnl et al., 2013). These anti-nutrient compounds and toxins are present in such low amounts that the safflower meal does not appear to be toxic when fed to animals.

17. High fibre content of the safflower seed or seed meal is the main factor limiting its use in livestock feed. Safflower petal extracts have been used in Chinese herbal medicine for centuries and there are many reports on the beneficial effects of safflower in the treatment of several conditions (Chengaiah et al., 2010; Zhou et al., 2014). Rare cases of allergic reactions to dried safflower flowers have been reported (Compes et al., 2006). There are also reports of adverse effects of high concentrations of safflower extracts in animal studies, indicating potential teratogenicity (Nobakht et al., 2000; Monfared, 2013), cytotoxicity (Mohseni et al., 2011) and nephrotoxicity (Liu et al., 2004).

4.1 Safflower as a crop

18. Safflower has been commercially cultivated as a minor crop in Australia since the 1950s. The growing area of safflower has fluctuated from year to year, with a peak of 75,000 hectares in 1979, which is less than 0.5% of the total cropping area in Australia. From 2004-2014, the average annual safflower planting area has ranged from 6,000-12,000 ha, this being mainly in New South Wales, Victoria and South Australia (ABARES, 2014).

19. Safflower is grown in Australia for the edible oil and industrial oil markets, but also whole safflower seeds are used for the birdseed market (GRDC, 2010). After oil is extracted from the seeds, the remaining meal can be used as stockfeed.

20. Cultivars of safflower are divided into two main classes. Linoleic safflower varieties have oil rich in linoleic acid (70-75%), while oleic safflower varieties have oil with high levels of oleic acid (70-80%) (Singh and Nimbkar, 2006). The GM safflower lines proposed for release were derived from the oleic type advanced breeding line M1582, obtained from Mexico. M1582 is not currently grown in Australia.

21. In Australia, oil derived from oleic safflower varieties is used for heat stable cooking oil, cosmetics and infant food formulations. Oil from linoleic safflower varieties is used for edible oil products such as salad oils and soft margarines (GRDC, 2010).

4.2 Weed risk potential for safflower outside cultivation

22. In the context of this RARMP, characteristics of safflower are examined when present as a volunteer in relevant agricultural land uses, in intensive use areas such as roadsides and in nature conservation areas.

23. *Carthamus tinctorius* L. is not recorded in the Australian government's Weeds of National Significance list (Department of Environment and Energy website, accessed 15 March 2018), the National Environmental Alert List (Department of Environment and Energy website, accessed 15 March 2018) or the *Noxious Weed List for Australian States and Territories* (Invasive Plants and Animals Committee, 2015). Safflower is naturalised in Australia and has been recorded as a weed of natural environments and of agricultural areas (Groves et al., 2003; Randall, 2007). In natural ecosystems safflower is considered a minor problem warranting control if present in 4 or more locations in a state or territory, and in agricultural settings volunteer safflower is considered a minor problem that does not warrant control (Groves et al., 2003).

24. The Standards Australia National Post-Border Weed Risk Management Protocol rates the weed risk potential of plants according to properties that correlate with weediness for each relevant land use (Standards Australia et al., 2006). These properties relate to the plants' potential to cause economic, environmental and/or social harm (impact); to spread, establish and reproduce (invasiveness); and to its potential distribution. The weed risk potential of volunteer safflower has been assessed using methodology based on the National Post-Border Weed Risk Management Protocol (OGTR, 2018).

4.2.1 Potential to cause harm

25. In summary, as a volunteer (rather than as a crop), non-GM safflower is considered to exhibit the following potential to cause harm:

- low potential to negatively affect the health of animals and/or people,
- low potential to reduce the establishment or yield of desired plants,
- low potential to reduce the quality of products or services obtained from all relevant land use areas,
- low potential to restrict the physical movement of people, animals, vehicles, machinery and/or water,
- low potential to act as a reservoir for a range of pests and pathogens,
- low potential to adversely affect soil salinity and the water table.

4.2.2 Invasiveness

26. With regard to invasiveness, non-GM safflower has:

- low ability to establish amongst existing plants,
- low tolerance to average weed management practices in cropping and intensive land uses,
- a short time to seeding (less than one year),
- low annual seed production,
- the ability to reproduce sexually, but not by vegetative means,
- low ability for long distance spread by natural means (wind/insect dispersal),
- high ability for long distance spread by people from dryland and irrigated cropping areas, but a low ability to spread from intensive land uses such as road sides,
- low ability for spread by people from or to nature conservation areas.

Section 5 The GM safflower – nature and effect of genetic modification

5.1 The genetic modification

27. The GM safflower lines proposed for release each contain an introduced gene silencing construct including fragments of two endogenous safflower fatty acid biosynthesis genes. The function of the silencing construct is to suppress the expression of these target genes, thus altering the oil composition of the GM safflower seeds. Lines expressing this silencing construct have been evaluated in previous RARMPs for limited and controlled release under the DIR licences 121 and 131.

28. The GM safflower lines also contain the selectable marker gene *hph*, derived from the soil bacterium *Streptomyces* sp. *Hph* encodes the hygromycin phosphotransferase enzyme, conferring resistance to the antibiotic hygromycin B. This marker was used in the laboratory to select transformed GM plants during early stages of development.

5.1.1 Details of the introduced genetic elements

29. The gene and gene fragments introduced into the GM safflower are listed in Table 1.

Table 1 Introduced gene and gene fragments in the GM safflower lines

Gene	Associated protein	Source organism	Function
CtFATB fragment	Palmytoyl-ACP thioesterase	*Carthamus tinctorius*	Fatty acid biosynthesis
CtFAD2.2 fragment	Δ12 desaturase	*Carthamus tinctorius*	Fatty acid biosynthesis
hph	Hygromycin B phosphotransferase (HPT)	*Streptomyces* sp.	Marker - antibiotic resistance (hygromycin)

30. Short regulatory sequences that control expression of the introduced genes are also present in the GM lines. These regulatory elements, derived from plants (flax, clustered yellowtops and castor bean), and micro-organisms (*Agrobacterium tumefaciens* and *Cauliflower mosaic virus*), are listed in Table 2.

31. Promoters are DNA sequences that are required in order to allow RNA polymerase to bind and initiate correct gene transcription. Expression of the silencing constructs are driven by the Flax linin seed-specific promoter (Patent US 7,642,346). Expression of the selectable marker gene *hph* is driven by a 35S promoter from the cauliflower mosaic virus (CaMV) (Kay et al., 1987), which leads to constitutive expression of the *hph* gene in all plant tissues.

32. Also required for gene expression in plants is a messenger RNA termination region (terminator), including a polyadenylation signal. The octopine synthase polyA (743bp) terminator and polyadenylation signal from *A. tumefaciens* (MacDonald et al., 1991) is used for the RNAi construct. The 3' non-translated

region of the nopaline synthase gene (239bp) terminator and polyadenylation signal from *A. tumefaciens* (Depicker et al., 1982; Bevan et al., 1983; Rogers et al., 1986) is used for the *hph* selectable marker.

33. The RNAi silencing cassette contains the two gene fragments in a hairpin arrangement separated by a 742bp PDK intron sequence (int1) from *Flaveria trinerva* (Wesley et al., 2001; Helliwell and Waterhouse, 2005) combined with 196bp of a Catalase 1 intron sequence (int2) from *Ricinus communis* (Wang et al., 1997; Helliwell and Waterhouse, 2005). The vector was constructed using the vector system described by Helliwell and Waterhouse (2005).

34. Although *A. tumefaciens* and CaMV are plant pathogens, and castor bean produces a toxin, the regulatory sequences comprise a small part of their total genome, and in themselves have no pathogenic, toxic or carcinogenic properties. All the source organisms for the introduced regulatory sequences are present in the Australian environment and thus humans and other organisms would commonly encounter them.

Table 2 Introduced regulatory elements in the GM safflower lines

Element	Function	Source
Flax linin promoter	Promoter used for gene silencing construct	*Linum usitatissinum* (flax)
Int1	Non-coding pyruvate dehydrogenase kinase intron sequence	*Flaveria trinervia* (clustered yellowtops)
Int2	Non-coding catalase 1 intron sequence	*Ricinus communis* (castor bean)
ocs	Terminator used for gene silencing construct (3' non translated region of the octopine synthase gene)	*Agrobacterium tumefaciens*
35S	Promoter used for selectable marker	*Cauliflower mosaic virus*
nos 3'	Terminator used for selectable marker (3' non translated region of the nopaline synthase gene)	*Agrobacterium tumefaciens*

5.1.2 Method of genetic modification

35. The GM safflower was produced using *Agrobacterium*–mediated transformation. This method has been widely used in Australia and overseas for introducing genes into plants. More information can be found in the document *Methods of Plant Genetic Modification* on the Risk Assessment References page on the OGTR website.

36. The parental variety used for transformation is the safflower advanced breeding line M1582. This is an oleic type advanced breeding line M1582, obtained from Mexico. M1582 is not currently grown in Australia. Transformed cells were selected on media containing an antibiotic hygromycin B. The antibiotic was also used to eliminate Agrobacterium during *in vitro* selection of the transformed safflower plants.

5.2 The introduced genes and fragments, their encoded proteins and associated effects

37. The two safflower genes that are targeted for suppression of expression are palmitoyl-ACP thioesterase (*CtFATB*) and Δ12 desaturase (*CtFAD2.2*). Suppression of the target genes is mediated using a natural regulatory mechanism in plants known as ribonucleic acid interference (RNAi) or gene silencing (Baykal and Zhang, 2010). Using the RNAi pathway, an introduced silencing construct is transcribed into double-stranded RNA, which is processed by endogenous cellular machinery into short interfering RNAs (siRNAs). The siRNAs direct the degradation of messenger RNA (mRNA) molecules with matching

sequence after the mRNAs are transcribed from genes and before they are translated into proteins. The efficiency of gene silencing is generally determined by the extent of homology between the silencing construct and the target gene (usually > 95% homology is required) and the length of the homologous region. In plants, introduced silencing constructs have been shown to effectively suppress expression of the target genes, but can also give rise to silencing of non-target genes with closely matching sequences.

5.2.1 The introduced gene sequences

38. The target gene *CtFATB* encodes a carrier protein that mediates export of saturated fatty acids from the plastid, where fatty acid synthesis occurs (Bonaventure et al., 2003). The effect of suppressing expression of *CtFATB* is to retain saturated fatty acids in the plastid until they undergo a desaturation reaction (usually to form oleic acid) and can be exported by another carrier protein. This decreases the proportion of saturated fatty acids and increases the proportion of oleic acid in the safflower oil (Wood et al., 2018).

39. The target gene *CtFAD2.2* encodes a desaturase protein that mediates enzymatic conversion of oleic acid to linoleic acid (Harwood, 1996). The effect of suppressing expression of *CtFAD2.2* is to decrease the proportion of linoleic acid and increase the proportion of oleic acid in the safflower oil (Wood et al., 2018).

40. The GM safflower lines produce seeds where approximately 92% of the total oil content is oleic acid (see Table 3). This high purity oleic oil has applications as an industrial raw material and a replacement for petroleum-based oils in the manufacture of plastics, lubricants and cosmetics (Vanhercke et al., 2013).

5.2.2 The hph gene and its products

41. The GM safflower lines also contain the introduced *hph* gene from *Streptomyces sp*. which encodes a hygromycin B phosphotransferase enzyme, providing resistance to the antibiotic hygromycin B. This antibiotic resistance trait was used as a selectable marker during plant transformation. Further information about this gene can be found in the document *Marker Genes in GM Plants* available from the Risk Assessment References page on the OGTR website.

5.2.3 Toxicity and allergenicity of the proteins encoded by the introduced genes and fragments

42. Insertion of gene fragments as part of the gene silencing construct does not result in expression of a new protein, but only in suppression of the expression of endogenous safflower proteins. This is not expected to lead to increased toxicity or allergenicity.

43. Bioinformatic analysis may assist in the assessment process by predicting, on a purely theoretical basis, the toxic or allergenic potential of a protein. The results of such analyses are not definitive and should be used only to identify those proteins requiring more rigorous testing.

44. The sequence similarity of the introduced protein fragments in the GM safflower lines against proteins with known or putative toxicity or allergenicity has been assessed. Sequence similarity search was conducted using the Food Allergy Research and Resource Program (FARRP) AllergenOnline search engine (Version 17, updated January 2017) and the UniProt database. All open reading frames (ORF) identified within the inserted gene construct (64 ORF for Event 26 and 62 for Event 40) were tested. None of the ORF within the introduced gene construct had immunological relevant similarities with any of the known or putative allergens or toxins in the databases.

45. The potential risks of the hygromycin B phosphotransferase (HPT) protein are discussed in the document *Marker Genes in GM Plants* available from the Risk Assessment References page on the OGTR website. There is no evidence that HPT is toxic or allergenic to humans.

5.2.4 Toxicity and allergenicity of oleic acid

46. The altered fatty acid composition of the GM safflower does not change the types of fatty acids found in safflower seeds, but only alters the ratios of palmitic, oleic and linoleic acids. These fatty acids

are not known to be toxic or allergenic and are present in most edible oils. Edible fatty acids collectively are an essential component of the diet. Current recommended levels of dietary fatty acids are 15-30% of total energy intake. More specifically, it is recommended that <10% of total energy comes from saturated fatty acids, 6 – 11% polyunsaturated fatty acids, <1% trans fatty acids and the remaining proportion comes from monounsaturated fatty acids (FAO, 2010).

47. The effect of the gene silencing is to increase levels of oleic acid and decrease levels of other fatty acids in GM safflower seed oil. Oleic acid is a common constituent of food, for example, it is the main constituent of olive oil and canola oil (Mailer, 1999; NSW DPI, 2006; RIRDC, 2007; NSW DPI, 2014; Calder, 2015). Increased oleic acid intake, particularly when used as a replacement for saturated fatty acids, has been shown to be beneficial to human health (Sales-Campos et al., 2013; Calder, 2015).

48. Safflower seed, meal and oil are not considered toxic, allergenic or pathogenic to humans or other organisms and have a long history of safe use (Cosmetic Ingredient Review, 1987, 2011; Toma et al., 2014). FSANZ has previously approved oil from GM high oleic soybean as safe for human consumption (FSANZ, 2009, 2011). The applicant has submitted an application to FSANZ to amend the Food Standards Code (Standard 1.5.2 – Food Produced Using Gene Technology) to include the GM safflower (A1156 - Food derived from Super High Oleic Safflower Lines 26 and 40).

5.3 Characterisation of the GMO

5.3.1 Molecular stability

49. A number of molecular analyses of both lines of the GM safflower were provided by the applicant. Southern blot analyses of digested DNA performed on T4 generation plants showed 1) the absence of unintended backbone sequences from the plasmids used for transformation and 2) that a single intact copy of the gene construct had integrated into each safflower genome. PCR-based genome walking analysis was also performed on T4 and T7 generation plants, confirming the presence of a stable, single copy insertion of the gene construct into each safflower genome.

50. Stability of the inserts in both lines was further examined by performing backcrosses of the GM safflower with non-GM safflower. Analysis of F2[3] seeds for fatty acid composition showed that both inserts were stable and inherited in a predictable manner, according to Mendelian principles.

5.3.2 Expression of the introduced construct

51. As discussed in Section 5.1 and 5.2, the GM safflower lines (Event 26 and Event 40) contain a construct designed to down-regulate two endogenous safflower fatty acid biosynthesis genes (*CtFATB* and *CtFAD2.2*). Down-regulation of these genes should lead to an increase in oleic acid (monounsaturated FA) and a decrease in linoleic acid (polyunsaturated FA) and palmitic acid (saturated FA).

52. The effect of the inserted gene construct on the GM safflower lines was investigated by the applicant by comparing the GM safflower lines with non-GM safflower varieties for

- lipid content and composition,
- transcription levels of the target genes and
- identification of small RNA populations.

53. Lipid composition of seed tissue and two-week old seedling tissue was assessed by Liquid Chromatography-Mass Spectrometry (LC-MS). Four non-GM safflower lines were used as controls:

- a low oleic non-GM safflower variety (LO)

[3] F1 plants were obtained by crossing the GM safflower lines with a non-GM line. F1 plants were then left to self-pollinate, generating F2 seeds that were used for analysis.

- two high oleic non-GM safflower varieties (HO1 and HO2)
- a high oleic non-GM safflower obtained by mutagenesis (HO/m)

Profiling of membrane-associated lipid species in various tissues across non-GM and GM safflowers, indicates that the effect of the genetic modification is restricted to seed and organs developmentally-derived from seed, such as the emergent cotyledons and hypocotyls (Figures 2 and 3) (Wood et al., 2018). The GM safflower lines showed a marked increase in monounsaturated fatty acids in seed tissues compared to non GM varieties LO, HO1 and HO2. In contrast, the lipid profile obtained in vegetative tissues was similar between the GM safflower lines and LO, HO1 and HO2 varieties for roots and true leaves, the dominant lipid species such as diacylglycerol (DAG), digalactosyldiacylglycerol (DGDG) and monogalactosyldiacylglycerol (MGDG) were unchanged between LO, HO, Event 26 and Event 40 and showed the high polyunsaturated fatty acid composition typical of these vegetative tissues. HO/m, obtained by mutagenesis, showed a marked increase in monounsaturated fatty acid content both in the seed and vegetative tissues compared to LO, HO1 and HO2 (Figures 2 and 3).

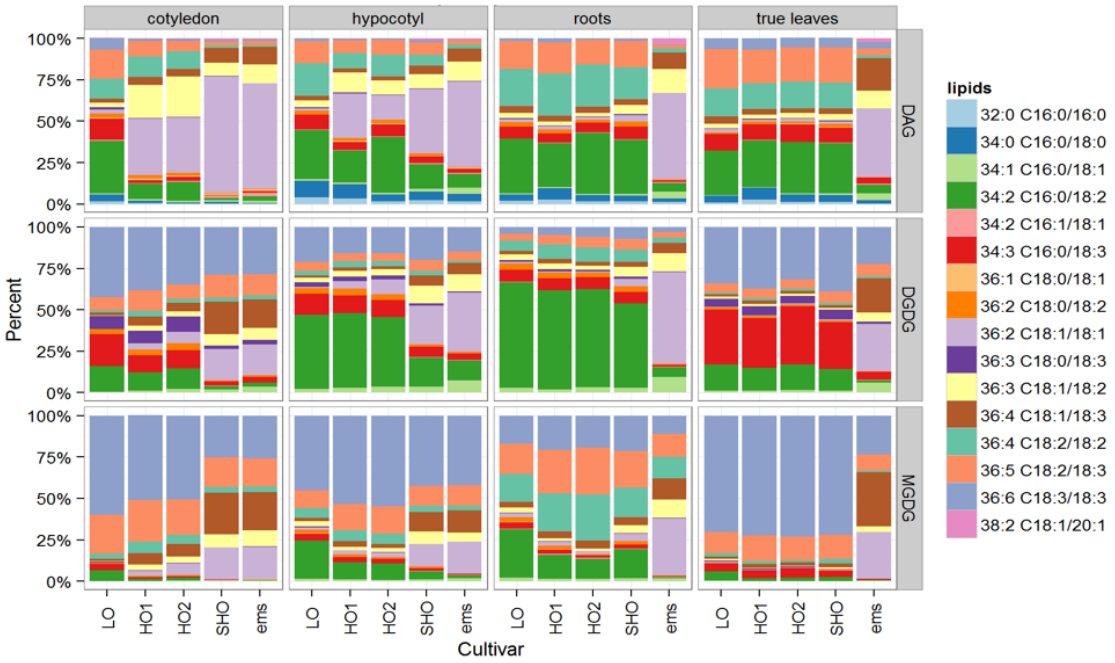

Figure 2. Profiling membrane-associated lipid species of different developmental stages from Event 26 and non-GM safflower varieties. The analysis includes varieties that have altered seed oleic profiles, such as low oleic (LO; Centennial), high oleic (HO1, S317; HO2, Lesaf496), super high oleic (SHO, Event 26) and S901 (ems). Other SHO lines were analysed and display similar trends for Event 26. Diacylglycerol (DAG), digalactosyldiacylglycerol (DGDG) and monogalactosyldiacylglycerol (MGDG).

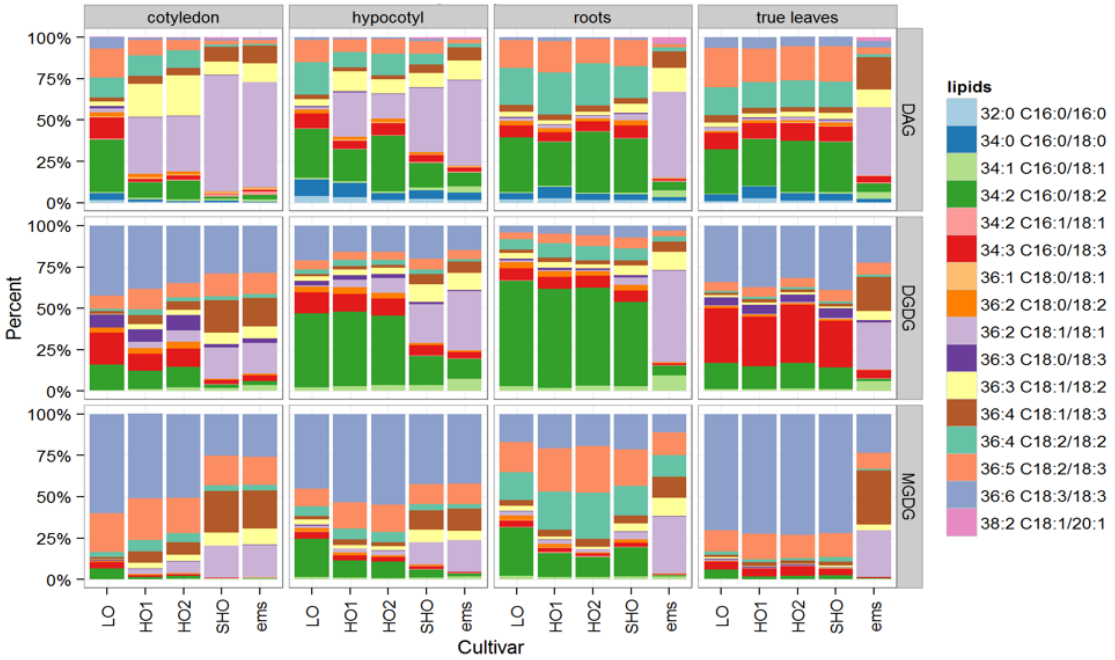

Figure 3. Profiling membrane-associated lipid species of different developmental stages from Event 40 and non-GM safflower varieties. The analysis includes varieties that have altered seed oleic profiles, such as low oleic (LO; Centennial), high oleic (HO1, S317; HO2, Lesaf496), super high oleic (SHO, Event 40) and S901 (ems). Other SHO lines were analysed and display similar trends for Event 40. Diacylglycerol (DAG), digalactosyldiacylglycerol (DGDG) and monogalactosyldiacylglycerol (MGDG).

54. Transcription levels of the target genes *CtFATB* and *CtFAD2.2* were measured in maturing safflower seeds using real-time quantitative PCR. Transcription levels for the GM safflower lines were compared to those of the high oleic non-GM variety HO1 mentioned above. Relative expression levels of both *CtFATB* and *CtFAD2.2* were significantly lower ($p<0.05$) than those of the non-GM HO1 variety. The expression level of *CtFATB* was significantly lower for Event 26 than it was for Event 40 ($p<0.01$). However, no significant difference was found between the two GM lines for *CtFAD2.2* ($p>0.05$).

55. Populations of small RNAs (sRNA)[4] were extracted from developing embryos of the GM safflower lines and deep sequenced[5]. Mapping of these sRNA populations showed that such sRNA only had significant matches to the sequences used in the RNAi gene silencing construct in both GM safflower lines. Expression of the RNAi gene silencing construct led to the production of sRNA targeting both *CtFATB* and *CtFAD2.2*.

56. Expression of the selectable marker *hph* gene was assessed by western blot analysis using specific anti-HPT antibodies. HPT was detected in the GM safflower lines, as a single protein product. The protein was not detected in the non-GM parental line.

5.3.3 Compositional analysis of GM safflower

57. The applicant provided compositional data for GM safflower seed, safflower meal and vegetative tissue. Plant material was harvested from field grown T4, T7 and T8 generation Event 26 and Event 40

[4] Plants produce a diverse population of small RNAs involved, among other things, in gene silencing.
[5] Deep sequencing refers to a sequencing aiming at a high number of replicate reads of a given genomic sequence. This leads to increased sequencing accuracy and allows for detection of rare variants.

plants. Field trials were conducted over three different locations, in NSW, Victoria and WA (Bellata, Kalkee and Kununurra, respectively) in 2014, 2016 and 2017 under licences DIR 121 and DIR 131.

58. Four non-GM safflower lines were used as controls:

- the parental line M1582
- a low oleic non-GM safflower variety (Sironaria)
- two high oleic non-GM safflower varieties (S-317 and Montola 2003).

59. The GM lines were grown as a block or as part of a randomised complete block design (RCBD)[6] together with the control lines. Fatty acid profiling and analysis of vegetative tissue was performed using samples grown in blocks, while seed compositional analysis and feed analysis was performed using samples grown in RCBD. Four parameters were studied:

- comparative analysis of seed nutritional composition
- impact of genetic modification on fatty acid composition of the seed
- comparative analysis of feed quality of safflower meal
- comparative analysis of feed quality of vegetative tissue.

60. Analysis of nutritional composition was performed using composite samples: seeds from different replicates of each line were pooled. Vegetative tissues were collected from bulk plantings.

61. Sixty-four analytes, classified in seven categories, were tested for nutritional compositional analysis of seed samples:

- proximates (crude protein content, moisture, fat, carbohydrates, ash and energy)
- vitamins (A, B1, B2, B3, B5, B6, B12, C, D2, folate and folic acid)
- minerals (Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn)
- total amino acids (alanine, arginine, aspartic acid and asparagine, glutamic acid and glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine)
- fatty acid profile and oil content
- total free sugar profile
- anti-nutrients (tannins and cyanide).

62. The nutritional analysis of seed from the two GM safflower lines showed that they were not significantly different to their non-GM safflower parental line and other non-GM safflower varieties for proximate content, vitamin composition, mineral content, amino acid composition, free sugar levels or anti-nutrient levels ($p>0.01$). Similarly, no significant difference was determined between the level of anti-nutritional tannins in safflower seed or vegetative tissues between Event 26, Event 40 and the parental line M1582 ($p>0.01$). All safflower varieties examined for hydrogen cyanide content of safflower seed or vegetative tissues, including Event 26, Event 40 and the parental line M1582, contained less than 2.5 ppm. This value is well below the values considered potentially toxic to grazing livestock (Landau et

[6] Randomised complete block design (RCBD) is the standard design for agricultural experiments. In RCBD, experimental units are grouped into blocks. Each block contains a complete set of treatments. This accounts for spatial effects and experimental error.

al., 2005; Williams, 2012). These results indicate that seeds from Event 26 and Event 40 are comparable to non-GM safflower seeds.

63. Fatty acid profile analysis of seed showed that the GM safflower lines are significantly different to their non-GM parental line for palmitic, oleic and linoleic acid content. The GM safflower lines showed decreased levels of palmitic and linoleic acids, and increased levels of oleic acid ($p<0.01$). This result was further supported in a separate experiment where the fatty acid composition of Event 26 and Event 40 seeds was compared with non-GM safflower lines that included high oleic types (M1582, S-317, Montola 2003) and a low oleic type (Sironaria) (Table 3). The GM safflower lines were comparable to the non-GM safflower lines except for oleic acid, linoleic acid and palmitic acid. Event 26 and Event 40 had very high levels of oleic acid (C18:1) and very low levels linoleic acid (C18:2) and palmitic acid (C16:0) compared to non-GM safflower. The proportion of monounsaturated and polyunsaturated fatty acids in the GM lines was significantly different to their non-GM parental line: the GM lines showed a marked increase in monounsaturated fatty acids and a marked decrease in polyunsaturated fatty acids. Oil stability[7] was significantly higher for the GM safflower lines compared to their non-GM parent. No significant difference was observed in oil viscosity or tocopherol levels.

Table 3 Fatty acid composition of safflower seed*

Fatty acid component	Event 26	Event 40	M1582 (high oleic type)	S-317 (high oleic type)	Montola 2003 (high oleic type)	Sironaria (low oleic type)
Palmitic acid (C16:0)	2.6	2.5	5.8	5.5	5.3	7.4
Oleic acid (C18:1)	92.0	92.3	75.2	67.8	74.8	11.8
Linoleic acid (C18:2)	1.2	1.6	14.7	21.9	16.1	76.3
Total fatty acids	100	100	100	100	100	100
Polyunsaturated	1.3	1.7	14.8	22.0	16.2	76.4
Monounsaturated	92.6	92.9	76.0	68.5	75.4	12.7
Saturated	6.1	5.4	9.2	9.5	8.4	10.9

*Fatty acid levels are presented as a percentage (%)

64. Several safflower seed products can be used as animal feeds, including as seeds for the bird market; and as meal for livestock (Oelke, 1992). Safflower meal, the by-product of oil extraction from safflower seeds, is mostly used as a protein ingredient for animal feeding (GRDC, 2010). Safflower meal produced from seeds with the hulls left on contains about 24% protein and high fibre content, while meal produced from seeds where most of the hulls are removed has about 40% protein (Oelke, 1992). Oil was extracted from composite safflower seed samples from the Bellata, NSW trial sites (DIR 131) and the resulting meal analysed for feed quality. Feed analysis included measurements of several parameters that are used to define the overall nutritive value. The data is presented on a dry matter basis (%, g/100g equivalent), unless otherwise stated. The results (Table 4) indicate that Event 26 and Event 40 safflower meal is comparable to the parental control line (M1582) and the non-GM safflower varieties tested.

[7] Oxidation stability of the oil was measured using the Rancimat method. This method is commonly used to measure the oxidation stability of vegetable and animal oils and fats and to examine the effectiveness of antioxidants.

Table 4 Feed analysis of safflower seed meal

Parameter	LOR *	Event 26	Event 40	M1582	Montola 2003	Sironaria
Neutral detergent fibre (%)	10	60	58	56	52	60
Acid detergent fibre (%)	2	43	41	39	36	41
Crude protein (%)	0.6	22.9	23.3	25.1	27.2	23.1
Crude fat (%)	0.5	7.0	7.8	8.1	10.0	7.8
Dry matter digestibility (%)	39	49	49	52	55	49
Digestible organic matter in the dry matter (%)	38	49	49	52	55	49
Inorganic ash (%)	1	3	3	3	4	3
Organic matter (%)	1	97	97	97	96	97
Metabolisable energy (MJ/kg DM)	4.3	9.6	9.8	10.2	11.2	9.7
Apparent metabolisable energy poultry (MJ/kg DM)	5.8	6.8	7.2	7.5	8.6	7.0

*LOR – 'limit of reporting', the minimum quantity that can be reported with confidence

65. The vegetative tissue from Event 26 (38 day old) and Event 40 (33 day old) were harvested and analysed for forage quality. Safflower can be grazed by sheep and cattle, and may provide valuable forage if it is harvested from mid-budding to early blooming stage (Landau et al., 2005). Safflower forage can also be valuable during drought, where seed remaining in the safflower stubble following harvest germinates after an early rainfall event, providing forage for livestock during autumn. The vegetative tissue from Event 26 and Event 40 were assessed for a number of parameters indicative of feed quality, and then compared to each other, as well as to values reported in scientific literature (Corleto et al., 2005; Serge Yan et al., 2005; Burhan Arslan, 2008; Peiretti, 2009; Danieli et al., 2011) for safflower vegetative tissue. The results indicate that there was no significant difference ($p>0.01$) between mean values for Event 26 and Event 40 with respect to vegetative feed quality (Table 5). In addition, the majority of the parameters assessed are similar to the non-GM safflower quoted in the reference literature (Table 5). The protein levels of Event 26 and Event 40 were much higher than reported in the literature; however proteins levels may vary depending on growth stage and agronomic conditions (Corleto et al., 2005; Peiretti, 2009; Danieli et al., 2011).

Table 5 Feed analysis of Event 26 and Event 40 vegetative tissue

Parameter	LOR	Event 26	Event 40	Literature range
Moisture (%)	0.5	89.75±0.05	90.45±0.35	91.7-97.0
Dry matter (%)	0.5	10.25±0.05	9.55±0.35	8.3-13.0
Neutral detergent fibre (%)	10	45.5±3.50	44.5±2.50	29.5-49.0
Acid detergent fibre (%)	2	23±1.00	21.5±1.50	17.2-35.0
Acid detergent lignan (%)	0.5	11.4±1.20	12.5±6.00	n/a
Crude fibre (%)	2	15.5±0.50	14.5±0.50	18.0-36.0
Crude protein (%)	0.6	39.25±0.95	40.4±0.70	7.0-27.0
Crude fat (%)	0.5	2.15±0.05	2.0±0.00	n/a
Organic matter (%)	1	86.5±0.50	86.5±0.05	82.9

Dry matter digestibility (%)	39	67.5±3.50	66.5±0.50	n/a
Digestible organic matter in the dry matter (%)	38	64.0±3.00	63.0±1.00	n/a
Inorganic ash (%)	1	13.5±0.50	13.5±0.50	12.0-17.0
Metabolisable energy (MJ/kg DM)	4.3	9.95±0.55	9.8±0.10	16.2

n/a – data not available, single values from (Peiretti, 2009)

66. In summary, the compositional data analysis supports the compositional equivalence of the GM safflower with non-GM safflower except for palmitic, oleic and linoleic acids. Other component values that were statistically significantly different between the GM and non-GM safflower lines represented differences that are not considered meaningful from a food or feed safety or nutritional perspective.

5.3.4 Phenotypic and agronomic characterisation

67. The phenotypic and agronomic performances of the GM safflower lines were assessed in field trials run in 2015 and 2016, under DIR 131. Field trials were conducted in NSW and Victoria over five different locations (Bellata, Wee Waa and Narrabri for NSW and Kalkee and Kaniva for Victoria) and two growing seasons.

68. The phenotypic characteristics measured represent characteristics that influence reproduction, crop survival and potential weediness. These characteristics were measured for Event 26 and Event 40, and compared to data obtained for the non-GM parent M1582. The characteristics measured were:

- Seedling vigour
- Plant height
- Time to flowering
- Disease incidence
- Insect damage
- Harvest lodging
- Capsule shattering
- Yield assessment

69. No significant differences were seen between the GM safflower lines and the parental lines for any traits except for plant height. Under some environmental conditions, Event 26 can be slightly shorter than Event 40 or M1582, the non-GM parental line. No pod shattering was observed in any safflower plots over all sites and years.

70. Seasonal and geographical variation in phenotypic characteristics was observed for both the GM and non-GM lines, with conditions observed in NSW in 2015 more favourable than these observed in Victoria. Significant variation between trial sites was found in 2015 and 2016 for seedling vigour, plant height, incidence of diseases, insect damage and yield. Significant variations in harvest lodging were found between trial sites in 2016 but not in 2015. Significant variations in time to flowering were found between trial sites in NSW on 2015 and 2016, as well as in Victoria in 2015. None of the variations were unusual and they were seen for both GM and non-GM lines.

71. In summary, GM safflower is similar to the non-GM safflower parent in agronomic performance. No unintended or pleiotropic[8] effects of the inserted genes have been identified.

Section 6 The receiving environment

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

73. The applicant has proposed to release the GM safflower lines Australia-wide. Therefore, for this licence application, it is considered that the receiving environment is all of Australia but in particular agricultural areas that are suitable to cultivate safflower. Commercial safflower production occurs in southern Queensland, NSW, Victoria and South Australia (GRDC, 2010). The actual locations, number of sites and area of land used in the proposed release would depend on factors such as field conditions, grower demand and seed availability.

6.1 Relevant agronomic practices

74. In Australia, safflower is an annual plant with a long growing season. It is generally sown in June or early July in northern and central NSW and during July in southern NSW, Victoria and South Australia. Provided there is water available, sowing could occur as late as September and early October in parts of Victoria and South Australia (OGTR, 2018). Safflower may be sown later than other winter crops, which allows it to be used for weed management or as an option when earlier planted winter crops have failed to establish (GRDC, 2010). The time from sowing to harvest is around 26–31 weeks, but varies with variety, location, sowing time and growing conditions. In Australia, flowering of winter sown safflower generally coincides with wheat harvest, and the crop is usually ready for harvest 4 to 6 weeks after wheat (GRDC, 2010). Harvest of safflower generally begins in late December in northern NSW and continues into March in the south east of South Australia. Safflower is generally harvested without swathing (OGTR, 2018).

75. It is anticipated that the agronomic practices for the cultivation of the GM safflower will not differ significantly from industry best practices used in Australia for non-GM and GM oilseeds. All safflower plants would be grown following standard safflower agricultural management practices and would receive seed treatments and applications of water, fertilisers, pesticides, and herbicides similar to current commercially grown non-GM safflower crops. Cultivation practices for safflower are discussed in more detail in *The Biology of* Carthamus tinctorius *L. (safflower)* (OGTR, 2018).

76. The applicant has developed a program to provide quality assurance and traceability in the production and supply of the GM safflower. This 'closed loop' identity preserved (CLIP) program will be used to establish contractual procedures between storage and handling operators and marketers, between transport operators, oilseed processors, seed companies and growers. The GM safflower would be kept separate from other safflower at all stages of supply, cultivation, processing and marketing. The applicant has also stated that propagation of GM safflower will be in accordance with the *Australian Seed Federation Best Practice Guidelines for the Management of GM Traits in Canola Seed* and the *Australian Oilseeds Federation (AOF) Market Choice Requirements for GM crops*.

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

6.2 Relevant abiotic factors

77. In Australia, safflower is an annual plant with a long growing season. It is best adapted to the cereal growing regions of southern NSW, Victoria and South Australia where there is higher rainfall (>450mm), a dry climate during late spring and early summer, and stored subsoil water reserves. It is relatively drought tolerant due to its extensive tap root system that can access moisture from deep in the soil profile. Safflower does have a relatively high water requirement but does not tolerate waterlogging, as this can starve roots of oxygen and encourage the development of the fungal diseases *Alternaria carthami* and *Phytophthora* species (GRDC, 2010).

78. Safflower seedlings at the rosette stage are resistant to cold and frosts as low as -7°C, but during stem elongation the growing point and stem can be damaged or killed by frosts below -4°C. Mean daily temperatures above 26°C during flowering and maturation reduce yield. Safflower can be grown in a range of soil types but prefers alkaline soils that are well drained (OGTR, 2018).

79. Other abiotic stresses that can reduce yield and oil content include susceptibility of young plants to hail damage (OGTR, 2018). Additional information regarding factors relevant to the growth, distribution and cultivation of commercial safflower in Australia can be found in *Raising the Bar with Better Safflower Agronomy* (GRDC, 2010) and *The Biology of* Carthamus tinctorius *L. (safflower)* (OGTR, 2018).

6.3 Relevant biotic factors

6.3.1 Presence of sexually compatible plants in the receiving environment

80. Safflower is primarily self-pollinating and cross-pollination rates have been found to be on average around 10% (Knowles, 1969). However, cross-pollination and seed set can be increased by insect pollinators (Claassen, 1950; Dajue and Mundel, 1996; GRDC, 2010), with wind-mediated outcrossing playing a minor role.

81. Safflower is grown as a minor commercial crop in Australia. An average of 10,000 hectares of safflower is grown annually in South Australia, New South Wales and Victoria (ABARES, 2014). Naturalised populations of wild safflower have been reported at low levels in all states and territories of Australia (Atlas of Living Australia). Wild safflower is considered a minor weed that primarily establishes on disturbed ground (Groves et al., 2003).

82. There are four related *Carthamus* species reported as present in Australia: *C. lanatus, C. leucocaulos*, *C. dentatus* and *C. glaucus.* All four species have a chromosome number of n=10, whereas for safflower n=12. These related species have all been reported as naturalised in Australia (Atlas of Living Australia). Both *C. lanatus* and *C. leucocaulos* have been declared noxious weeds in some states or territories (Weeds Australia). There are doubts about the existence of *C. glaucus* in Australia; the two specimens that formed the basis of the record of this species in the 1986 Flora of SA have now been re-determined as *C. leucocaulos*, and the same may have happened in other States (personal communication Micheala Heinson, PIRSA, SA government). Under controlled conditions, *C. leucocaulos* and *C. lanatus* can cross with *C. tinctorius* but produce sterile F1 hybrid plants (Mayerhofer et al., 2011). One study of crosses between *C. tinctorius and C. glaucus* produced fertile offspring under controlled conditions, but doubts have been raised about the identity of *C. glaucus* seeds supplied (Mayerhofer et al., 2011), whereas another study indicated hybrids with *C. glaucus* are sterile (Ashri and Knowles, 1960). Similar to the other n=10 species above, formation of viable hybrids between *C. dentatus* and safflower (n = 12) is unlikely due to different chromosome numbers (Kumar, 1991; McPherson et al., 2004).

6.3.2 Presence of other biotic factors

83. In Australia, the main insect pests of safflower are aphids (plum, green peach, leaf curl), cutworms (*Agrotis* spp.), native budworm or heliothis (*Helicoverpa* spp.), rutherglen bugs (*Nysius vinitor*), red-legged earth mites (*Halotydeaes destructor*) and blue oat mite (*Penthaleus major*). These pests can all be controlled with insecticides and some with biological controls (GRDC, 2010). Other pests known to infest safflower crops in Australia include thrips, Lucerne flea, black field crickets, grasshoppers, locusts,

wireworms, false wireworms, jassids and myrids (GRDC, 2010). Safflower is most susceptible to damage by insects during establishment and between budding and harvest (GRDC, 2010).

84. Beneficial insects such as ladybirds and spiders have also been found in Australian safflower fields (GRDC, 2010). Pests such as pigs and kangaroos are deterred from grazing safflower by its spines and unpalatability. Bird damage can be an issue especially when safflower is grown near forested areas which harbour birds (GRDC, 2010).

85. A number of diseases can infect safflower, especially in warm and humid conditions. Diseases are more prevalent under irrigation conditions than if rain-fed (Nimbkar, 2008). The three main diseases of safflower in Australia are the fungal diseases Alternaria blight (*Alternaria carthami*), Phytophthora root rot (*Phytophthora cryptogea*) and rust (*Puccinia carthami*). Other less prevalent diseases in Australia include seedling damping off, grey mould, charcoal rot, leaf spot and sclerotinia (GRDC, 2010). At present there are no fungicides registered for disease control in safflower in Australia (GRDC, 2010)[9]. Control of disease in Australia relies on using appropriate crop rotations, selecting resistant varieties, using clean seed, controlling volunteer and weed hosts, sound irrigation practices and selecting appropriate soils. The fact that safflower is a minor crop is an important contributor to reduced disease incidence (OGTR, 2018).

86. Weeds that compete with safflower include grass and broadleaf weeds. Safflower is a poor competitor with weeds, due to slow growth at the rosette stage early in the season (GRDC, 2010). Weed management is therefore essential when growing this crop. Later in the season many weeds can outgrow safflower in height and the resulting shading can reduce crop yields significantly (Dajue and Mundel, 1996; GRDC, 2010). Safflower can be sown later than other winter crops which enables more time for control of weeds with knockdown herbicides or cultivation prior to sowing (GRDC, 2010). Safflower is tolerant of some herbicides, but as a minor crop in Australia, the number of herbicides available for use in Australia is limited (GRDC, 2010). Several pre-emergent herbicides, as well as in-crop grass and broadleaf selective products are registered for use in safflower (OGTR, 2018). Cultivation when the safflower plants are 7 to 15 cm tall can also be used to control small, later germinating weeds (GRDC, 2010).

6.3.3 Use of hygromycin B in agriculture and medicine

87. Internationally, hygromycin B is used in animal production as a feed additive for swine and chickens to kill parasitic worms, e.g. in Hygromix® products registered by the U.S. Food & Drug Administration (US FDA website, accessed 24 November 2017). Hygromycin B is currently not registered for use as a veterinary medicine in Australia (APVMA PubCRIS database, accessed 5 July 2017) and is not on the international *OIE List of Antimicrobial Agents of Veterinary Importance* (OIE, 2015).

88. Hygromycin B is not used in human medicine in Australia and is currently not listed in the Australian Register of Therapeutic Goods (TGA website, accessed 24 November 2017). Furthermore, the antibiotic is not considered high priority for managing the development of antibiotic resistance: it is not listed in the Australian Strategic and Technical Advisory Group on Antimicrobial Resistance's *Importance Ratings and Summary of Antibacterial Uses in Humans in Australia* (ANSTAG, 2015) or the *World Health Organization list of Critically Important Antimicrobials for Human Medicine* (WHO, 2017).

89. In addition to hygromycin B, the HPT protein phosphorylates the closely related compounds hygromycin B_2, destomycin A and destomycin B (Rao et al., 1983; FSANZ, 2006). These compounds are not generally used in human or veterinary medicine.

[9] The Australian Oilseeds Federation and NSW-DPI both indicated that as of Nov 2014, there were no permits from APVMA for use of fungicides on safflower.

6.4 Presence of the introduced genes and encoded proteins in the receiving environment

90. The two gene fragments included in the silencing constructs are from endogenous safflower genes that are naturally present in all safflower plants. The *hph* antibiotic resistance gene is from the soil bacterium *Streptomyces* sp. which is widespread and prevalent in the environment.

Section 7 Previous authorisations

7.1 Australian authorisations

91. The Regulator has approved field trials of these GM safflower lines, and other safflower lines with the trait of increased oleic acid, under licences DIR 121 and DIR 131.

92. The Regulator has previously approved field trials of GM cotton with the trait of increased levels of oleic acid under licences DIR 039/2003 and DIR 085/2008.

93. Information on these licences is available from the GMO Record on the OGTR website. There have been no reports of adverse effects on human health or the environment resulting from any of these releases.

7.2 Approvals by other Australian agencies

94. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. However, dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products.

95. FSANZ is responsible for human food safety assessment and food labelling, including GM food. FSANZ has previously approved food derived from lines of GM soybean with the trait of increased levels of oleic acid as safe for human consumption (FSANZ, 2009, 2011). An application to FSANZ for approval of their GM safflower lines was lodged by GO Resources Pty Ltd on 4 January 2018.

7.3 International authorisations

96. None of the GM safflower lines proposed for release in this application have been approved for release in other countries.

97. Field trials of different GM safflower lines, with introduced traits other than altered oil content, have been approved in the United States and Canada (USDA-APHIS, 2008; CFIA, 2015).

Chapter 2 Risk assessment

Section 1 Introduction

98. 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 context established for the risk assessment (see Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

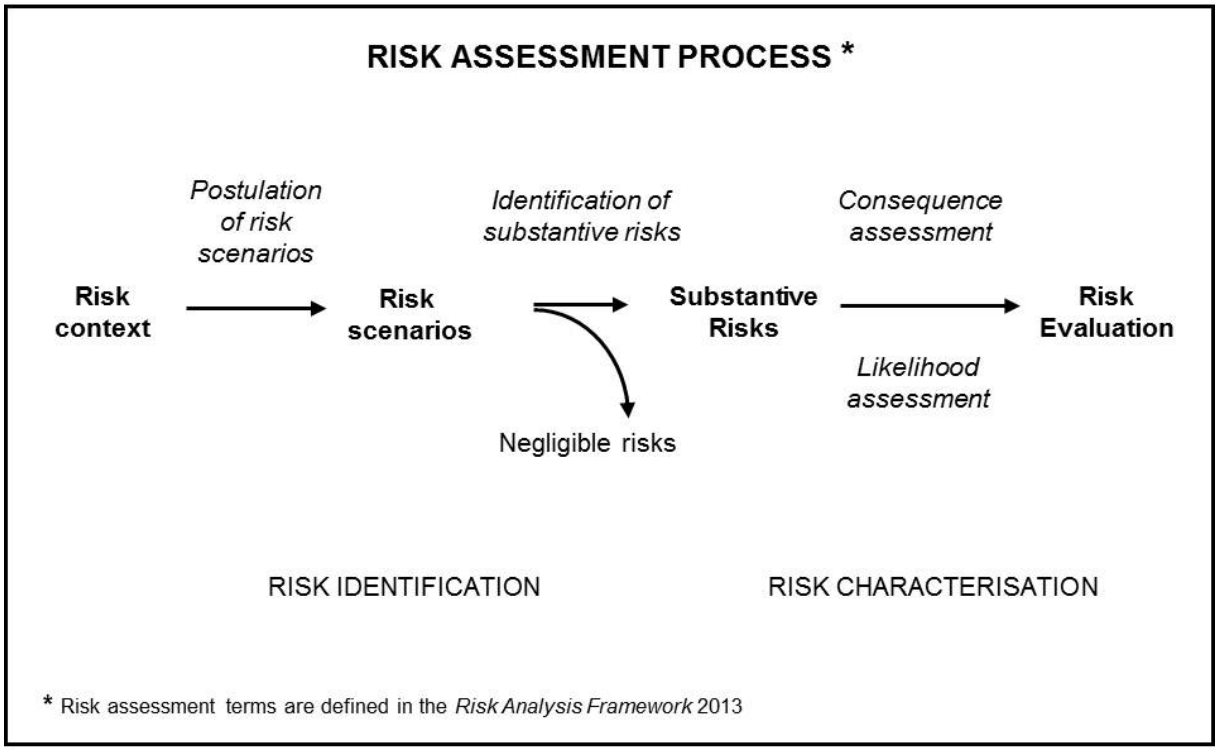

Figure 4 The risk assessment process

99. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO in the short and long term. These are called risk scenarios.

100. A number of risk identification techniques are used by the Regulator and staff of the OGTR, 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 considered in postulating risk scenarios (Keese et al., 2014). Risk scenarios postulated in previous RARMPs prepared for licence applications for the same or similar GMOs are also considered.

101. Postulated risk scenarios are screened to identify those that are considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

102. Substantive risks (i.e. those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). Risk evaluation then combines the Consequence and Likelihood assessments to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 Risk identification

103. Postulated risk scenarios are comprised of three components:

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.

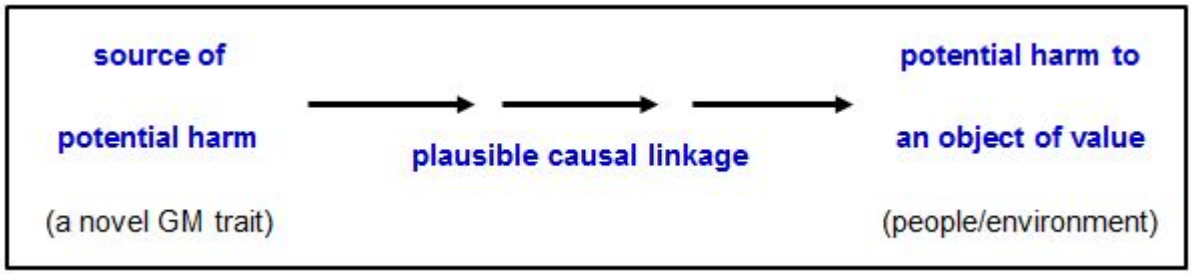

Figure 5 Risk scenario

104. When postulating relevant risk scenarios, the risk context is taken into account, including the following factors:

- the proposed dealings, which may be to conduct experiments, develop, produce, breed, propagate, grow, import, transport or dispose of the GMOs, use the GMOs in the course of manufacture of a thing that is not the GMO, and the possession, supply and use of the GMOs in the course of any of these dealings
- any proposed limits including the extent and scale of the proposed dealings
- any proposed controls to restrict the spread and persistence of the GMOs
- the characteristics of the parent organism(s).

2.1 Risk source

105. 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.

106. As discussed in Chapter 1, Section 5.1.1, the GM safflower proposed for release has been modified by the introduction of an RNAi gene silencing construct targeting the two endogenous safflower genes *CtFATB* and *CtFAD2.2*, involved in fatty acid biosynthesis. This introduced gene construct is considered further as a potential source of risk.

107. The GM safflower also contains the *hph* antibiotic resistance selectable marker gene, where the encoded protein, HPT, inactivates the antibiotic hygromycin B. The *hph* gene and its product have already been extensively characterised and assessed as posing negligible risk to human or animal health or to the environment by the Regulator as well as by other regulatory agencies in Australia and overseas. Further information about this gene can be found in the document *Marker Genes in GM Plants* available from the Risk Assessment References page on the OGTR website. As this gene has not been found to pose a substantive risk to either people or the environment, its potential effects will not be further considered for this application.

108. The introduced gene construct contains regulatory sequences that are necessary for expression of the silencing sequences and the selection marker gene. These regulatory sequences are derived from flax, castor bean, a common soil bacterium and a plant virus (Chapter 1, Section 5.1.1). Regulatory sequences are naturally present in plants, and the introduced elements are expected to operate in similar ways to endogenous elements. The regulatory sequences are DNA that is not expressed as a protein, and dietary DNA has no toxicity (Society of Toxicology, 2003). As described in Chapter 1, these types of sequences have been widely used in other GMOs, including in GM plants such as cotton or canola grown commercially in Australia and overseas without reports of adverse effects. Hence, potential risks from the regulatory elements will not be considered further.

109. The genetic modification has the potential to cause unintended effects in several ways including altered expression of endogenous genes by random insertion of introduced DNA in the genome, increased metabolic burden due to modification of endogenous pathways, 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 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). No biologically significant differences were found in the biochemistry, physiology or ecology of the GM safflower lines, when compared with non-GM safflower (Chapter 1, Section 5.3), and the introduced constructs are stable (Chapter 1, Section 5.3.1). Therefore, unintended effects resulting from the process of genetic modification will not be considered further.

2.2 Causal pathway

110. 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 GM plants (e.g. reproductive characteristics, dispersal pathways and establishment potential)
- tolerance to abiotic conditions (e.g. climate, soil and rainfall patterns)
- tolerance to biotic stressors (e.g. pests, pathogens and weeds)
- tolerance to cultivation management practices
- gene transfer to sexually compatible organisms
- gene transfer by horizontal gene transfer
- unauthorised activities.

111. Although all of these factors are taken into account, some are not included in risk scenarios because they are regulated by other agencies, have been considered in previous RARMPs or are not expected to give rise to substantive risks (see Sections 2.2.1 to 2.2.3, below).

2.2.1 Tolerance to abiotic factors

112. The geographic range of non-GM safflower in Australia is limited by a number of abiotic factors including climate and soil compatibility, as well as water and nutrient availability (OGTR, 2018, 2016). The introduced RNAi gene silencing construct is unlikely to make the GM safflower plants more

tolerant to abiotic stresses that are naturally encountered in the environment and is therefore unlikely to alter the potential distribution of the GM safflower plants. Also, as discussed in Chapter 1, Section 5.3, there was no consistent significant difference between the GM safflower lines and non-GM safflower varieties in response to abiotic factors. Therefore, tolerance to abiotic stresses will not be assessed further.

2.2.2 Horizontal gene transfer

113. The potential for horizontal gene transfer (HGT) and any possible adverse outcomes has been reviewed in the literature (Keese, 2008) and assessed in previous RARMPs. No risk greater than negligible was identified, due to the rarity of HGT events and because the gene sequences (or sequences which are homologous to those in the current application) are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, HGT will not be assessed further.

2.2.3 Unauthorised activities

114. Previous RARMPs have considered the potential for unauthorised activities to lead to an adverse outcome. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires the Regulator to have regard to the suitability of the applicant to hold a licence prior to the issuing of a licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities, and no risk greater than negligible was identified in previous RARMPs. Therefore unauthorised activities will not be considered further.

2.3 Potential harm

115. Potential harms from GM plants include:

- harm to the health of people or desirable organisms, including toxicity/allergenicity
- reduced biodiversity for nature conservation
- 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).

116. These harms are based on those used to assess risk from weeds (Standards Australia et al., 2006; Keese et al., 2014). Judgements of what is considered harm depend on the management objectives of the land where the GM plant may be present. For example, 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

117. Four risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 6 and discussed in depth in Sections 2.4.1 to 2.4.3. Postulation of risk scenarios considers impacts of the GM safflower or its products on people undertaking the dealings, as well as impacts on people and the environment exposed to the GM safflower or its products as the result of commercial use or the spread and persistence of plant material.

118. In the context of the activities proposed by the applicant and considering both the short and long term, none of the four risk scenarios gave rise to any substantive risks that could be greater than negligible.

Table 6 Summary of risk scenarios from the proposed dealings

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced gene construct for high oleic acid composition.	Commercial cultivation of GM safflower expressing the introduced gene construct. ⬇ Exposure of people and other organisms by contact, ingestion, or inhalation.	• Increased toxicity or allergenicity to people. • Increased toxicity to other organisms.	No	• Insertion of the silencing constructs does not lead to expression of a protein. • The biosynthetic pathway that is the target of the genetic modification does not produce known toxins or allergens.
2	Introduced gene construct for high oleic acid composition.	Dispersal of GM safflower outside intended cropping areas. ⬇ Establishment of populations of volunteer GM plants.	• Increased toxicity or allergenicity in people or toxicity to other organisms. • Reduced establishment of desirable vegetation. • Reduced quality of the biotic environment.	No	• Insertion of the silencing constructs does not lead to expression of a protein. • The biosynthetic pathway that is the target of the genetic modification does not produce known toxins or allergens. • The genetic modifications are not expected to increase the ability of the GM plants to spread and persist.
3	Introduced gene construct for high oleic acid composition.	Commercial cultivation of GM safflower producing seed containing high oleic acid. ⬇ GM safflower seed consumed by pest animals. ⬇ Increased fitness of pest animals. ⬇ Impact of these animals on native or desirable vegetation.	• Reduced establishment of desirable vegetation. OR • Reduced biodiversity.	No	• Exposure of pest animals and insects to high oleic acid in the GM safflower seed is limited. • A small increase in oleic acid in the diet is unlikely to increase pest animal fitness beyond that of a normal abundant diet. • Pests are controlled by current pest management practices.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
4	Introduced gene construct for high oleic acid composition.	Commercial cultivation of GM safflower expressing the introduced gene construct. ⬇ Dispersal of GM safflower outside intended cropping areas. ⬇ Vertical transfer and expression of introduced genes in other sexually compatible plants (eg. weedy relatives or commercial varieties of safflower). ⬇ Establishment of populations of volunteer GM plants.	• Increased toxicity or allergenicity in people or toxicity to other organisms. • Reduced establishment or yield of desirable vegetation. • Reduced biodiversity.	No	• Safflower does not produce fertile hybrids with related weedy species in Australia. • The introduced genes are not expected to increase the ability of hybrid GM plants to spread and persist or to be toxic or allergenic.

2.4.1 Risk scenario 1

Risk source	Introduced gene construct for high oleic acid composition.
Causal pathway	⬇ Commercial cultivation of GM safflower expressing the introduced genes. ⬇ Exposure of people and other organisms by contact, ingestion, or inhalation. ⬇
Potential harm	Increased toxicity or allergenicity to people. OR Increased toxicity to other organisms.

Risk source

119. The source of potential harm for this postulated risk scenario is the introduced RNAi gene silencing construct.

Causal pathway

120. The silencing constructs with safflower gene fragments are designed to produce siRNAs that suppress the expression of fatty acid biosynthetic genes, thus altering seed oil fatty acid content. A range of organisms may be exposed directly or indirectly to the introduced genetic constructs or their end products. Workers cultivating the GM safflower would be exposed to all plant parts. People involved in the breeding, cultivating, harvesting, transporting and processing of the GM safflower may be exposed to its products through contact (including inhalation of pollen). This would be expected to mainly occur in the cropping areas, but could also occur anywhere the GM plant material was transported or used. The GM plant material is proposed for industrial oil production, not for

human food. Therefore, people are unlikely to be exposed to the introduced genes or their end products as a result of consuming safflower oil or seed.

121. Organisms, including birds, rodents and invertebrates, may be exposed directly to GM safflower plants through biotic interactions (vertebrates, invertebrates, symbiotic and/or pathogenic microorganisms), through contact with dead plant material or indirectly through the food chain. Due to the spiny nature of the plant, large animals do not generally graze safflower. However, meal derived from crushing of the seeds would be used as livestock feed. Livestock would therefore be exposed to products derived from the GMOs.

Potential harm

122. The introduced gene silencing construct could lead to production of substances in the GM safflower that are toxic or allergenic for people or toxic for other organisms.

123. Transcription of the RNAi gene silencing construct leads to the production of hairpin RNA. This double-stranded RNA enters the RNAi pathway rather than being translated into a protein. Therefore, the introduction of the silencing constructs does not lead to expression of a novel protein that could potentially be toxic or allergenic. All known allergens are proteins, with the main sources of plant allergens being peanut, tree nuts, wheat and soybean (Delaney et al., 2008; Herman and Ladics, 2011).

124. Humans and animals have a long history of safe exposure to non-GM safflower. Seed and oil extracted from seed are the primary part of safflower used as human food and animal feed (AOSCA, 2012; Pearl et al., 2014). Non-GM safflower seed has a history of safe use in human and animal diets. Safflower seed oil is non-allergenic and is suitable for use in injectable medicines and cosmetics (Smith, 1996).

125. The plant is also used in Chinese alternative medicine, with different usages for leaves, flowers or seeds. Flowers are also occasionally used as a cheap substitute for saffron. Rare cases of allergic reactions to safflower dried flowers have been reported (Compes et al., 2006). There is only one reported case of an allergenic reaction to safflower dried flowers in humans (Compes et al. 2006) and there are some reports of adverse toxic effects of safflower floral extract injections used in Chinese alternative medicine (see Chapter 1, Section 4). However, it is not clear whether the adverse reaction is due to safflower flower extract or to other liposoluble components (Zhang et al., 2009).

126. The two genes targeted by the RNAi gene silencing construct are *CtFATB* and *CtFAD2.2*. These two genes, described in Chapter 1 Section 5.2, are involved in fatty acid biosynthesis, with their suppression leading to increased oleic acid content and decreased palmitic and linoleic acid content in the seed. Oleic acid is described as the most abundant fatty acid, and is a major constituent of vegetable oils (such as olive oil, canola oil, sunflower oil or peanut oil) and animal fats (such as beef tallow, pork lard or poultry fat) (AOCS Lipid library). Oleic acid is the principal fatty acid in the Western diet and is not considered toxic or allergenic (Arab, 2003).

127. Introduction of the RNAi construct could potentially lead to off-target siRNA-mediated silencing of endogenous safflower genes. However, no phenotypic, compositional or agronomic variation was observed for the GM safflower lines compared to their non-GM parents, apart from the intentionally altered palmitic, oleic and linoleic acid content in the seed as described in Chapter 1, Section 5.3. The only phenotypic difference seen between GM safflower and non-GM safflower is altered fatty acid composition in the GM safflower oil. Compositional data analysis of GM safflower from field trials conducted under licences DIR 121 and DIR 131 supports the compositional equivalence of the GM safflower with non-GM safflower except for palmitic, oleic and linoleic acids (Chapter 1, Section 5.). The GM safflower lines showed decreased levels of palmitic and linoleic acids, and increased levels of oleic acid.

128. In these circumstances, there is no reasonable expectation that the introduced constructs will lead to an increase in the level of any endogenous compound in the GM safflower that has toxic or allergenic properties.

129. Hairpin RNA transcribed from the silencing constructs is processed into siRNAs. siRNAs fall under a general category of small RNAs (sRNAs) that also includes microRNAs (miRNAs). siRNAs and miRNAs are common in both plants and animals and are believed to play regulatory roles in many biological processes. Animals and plants naturally produce thousands of different siRNA molecules and these are consumed by humans and other organisms whenever they eat plant or animal cells (Petrick et al., 2013). One paper (Zhang et al., 2011) tracked the metabolic fate of a particular natural miRNA, miR-168a, that is produced abundantly in rice and other plants and happens to have a near perfect sequence match to a mammalian gene. In a study of mice fed a pure rice meal after fasting, the plant miRNA was detected in mouse livers and was reported to modulate the expression of the matching mammalian gene, reducing levels of the encoded protein in the liver by approximately 50%. However, a large quantity of rice was fed to the mice in the Zhang et al study (2011), equivalent to a human eating approximately 33 kg/day of cooked rice, an unrealistic quantity in any human diet. The reported effect on the mouse gene by the plant miRNA was transient and ceased when rice was no longer included in the food intake. An analysis paper (Petrick et al. 2013) suggests some potential alternate explanations for the findings of the Zhang et al study (2011), and after reviewing a number of other papers in the field concluded that the weight of the evidence does not suggest that miRNAs derived from normal dietary exposure have a meaningful effect on mammalian gene expression.

130. FSANZ (FSANZ, 2013) also reviewed the scientific literature of research into RNAi mechanisms across different species and in different biological contexts in response to an article on biosafety risks from gene silencing mechanisms (Heinemann et al., 2013). FSANZ concluded that the weight of scientific evidence published did not support the view that small dsRNAs in foods are likely to have adverse consequences for humans (FSANZ, 2013). Furthermore, the current case-by-case approach to GM food safety assessment is sufficiently broad and flexible to address GM food safety (FSANZ, 2013; Casacuberta et al., 2015).

131. Further studies, analysing animal tissues and fluids, or feeding experiments, have found that sRNAs from dietary sources can be observed in mammalian tissues and dietary material, but disagree on whether the level detected is biologically significant or technical artefact (Dickinson et al., 2013; Witwer et al., 2013; Beatty et al., 2014; Liang et al., 2014; Bagci and Allmer, 2016; Chin et al., 2016). A comprehensive meta-study of sRNAs by Kang et al. (2017) combined the analysis of sRNAs in 824 datasets from human tissues and body fluids with controlled feeding experiments in rats and piglets. The results of this meta-analysis indicated that the dietary sRNAs detected were from technical artefacts rather than dietary intake (Kang et al., 2017). Their conclusions are further supported by a recent review paper by Chan and Snow (2017) which examined data from different studies using dietary delivery of sRNA in mammals, as well as studies of sRNA function in mammals, invertebrates and plants. Assessment of this data found that dietary material does not contain enough sRNA to allow the uptake of biologically meaningful levels in mammals; there was low ability of ingested sRNAs to survive the mammalian digestive tract; no known mechanisms for the transfer of sRNAs across the epithelium of the human digestive tract at the molecular level; and uncertainty about systemic spread and uptake of sRNAs between cells (Chan and Snow, 2017). The authors concluded that there is no decisive proof supportive of the dietary uptake of sRNAs at biologically relevant levels (Chan and Snow, 2017).

132. The possibility exists that siRNAs produced in GM safflower lines could modulate expression of human or animal genes, with unknown physiological effects. The siRNAs would need to be produced at high levels in GM safflower, a large amount of the GM safflower would need to be consumed, the siRNA would need to match a target sequence in a human or animal gene, and be taken up by cells expressing that gene. Mammals do not have genes that are homologous to the safflower fatty acid

biosynthesis genes targeted by the introduced silencing constructs. Even if siRNAs were acquired through eating GM safflower and did affect expression of a mammalian gene, it is expected that any effect would be transient as described in Zhang et al (2011).

133. RNAi technology has been used to develop a number of GM crops that have been approved for use as food, feed or cultivation in a number of countries (SCI 2017). GM alfalfa that has been modified using RNAi for altered lignin production has been approved for use as feed in Australia, Japan, Mexico, Singapore and South Korea. GM potato that has been altered using RNAi to reduce the potential for acrylamide production has also been approved for food in Australia and New Zealand (FSANZ, 2016), for feed in South Korea, and for food and feed in Malaysia. GM soybean that has been modified using RNAi for high oleic acid composition has been approved for food in a number of countries including Australia, New Zealand, European Union and Mexico. All of these GM crops, as well as GM apple modified for non-browning, have been approved for commercial release in Canada and the USA (ISAAA database). There are no known reports of adverse effects from the release of these GM crops.

134. Herbivorous insects that feed on the GM safflower could ingest the sRNAs, leading to off-target gene silencing. This could have adverse effects on populations of invertebrates that feed on GM safflower. The uptake of dietary sRNAs was first described in *Caenorhabditis elegans*, where dietary sRNAs were found to silence multiple genes after serving as the template for the formation of sRNA (Timmons and Fire, 1998; Timmons et al., 2001). Since then, many studies have shown that RNAi can be used in pest control (Mamta and Rajam, 2017). A number of studies have shown that there is species-dependant variability in the ability of invertebrates to take up dietary sRNAs from different dietary sources (Dowling et al., 2016; Chan and Snow, 2017). Lepidopteran insects have low RNAi efficiency, while Orthoptera, Coleoptera and Hemiptera are more sensitive to RNAi, although there is variability in these Orders as well (Guan et al., 2018). In bees, ingested sRNAs from pollen are not efficiently taken up by the digestive tract or dispersed to other tissues under normal conditions (Masood et al., 2016). The RNAi insensitivity in insects could be related to several factors, including an impaired cellular uptake, the presence of viral infections and saturated RNAi machinery (Christiaens et al., 2014). The degradation of dsRNA in the invertebrate digestive system appears likely to play a major role in this low sensitivity to RNAi (Guan et al., 2018). It is not known if invertebrates feeding on the GM safflower will be insensitive to the RNAi. There are no known reports of adverse effects on invertebrate populations resulting from currently approved GM crops using RNAi technology.

Conclusion

135. Risk scenario 1 is not identified as a substantive risk, due to the introduced gene fragments not coding for any proteins and the biosynthetic pathway they affect being involved in the production of compounds that have not been associated with toxic or allergenic reactions. 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 gene construct for high oleic acid composition.
Causal pathway	Dispersal of GM safflower outside intended cropping areas. ⬇ Establishment of populations of volunteer GM plants.
Potential harm	Increased toxicity or allergenicity in people or toxicity to other organisms. OR Reduced establishment of desirable vegetation. OR Reduced quality of the biotic environment.

Risk source

136. The source of potential harm for this postulated risk scenario is the introduced RNAi gene silencing construct.

Causal pathway

137. Dispersal of GM safflower seed outside intended cropping areas could result in the germination and subsequent growth of GM plants expressing the introduced gene construct. These plants could spread and persist in the environment outside cropping areas.

138. Baseline information on the weediness of safflower is given in Chapter 1, Section 6, and more detailed consideration is available in *The Biology of* Carthamus tinctorius *L. (safflower)* (OGTR, 2018). Safflower is fairly slow-growing, with an extended rosette stage following emergence and prior to stem development, during which it is poorly competitive with other plants (Dajue and Mündel, 1996). Safflower plants are susceptible to a wide range of herbicides as well as physical weed management practices (GRDC, 2010).

139. Potential dispersal of reproductive GM plant material outside intended cropping areas would be limited to seed or pollen, as safflower does not reproduce vegetatively in the field. Safflower seed heads are resistant to shattering and the seeds lack seed dispersal characteristics such as stickiness, burrs and hooks, which can contribute to seed dispersal via animal fur or feathers. These seed dispersal characteristics are not expected to be altered in the GMOs. Gene flow via pollen is discussed in Section 2.4.3.

140. Dispersal of viable seed could occur through a variety of ways including: endozoochory (dispersal through ingestion by animals), transport of seeds by animals, movement of seeds by people, or extremes of weather such as flooding or high winds. Seeds dispersed by flooding would be unlikely to survive and establish, as safflower is very susceptible to damping off and fungal diseases in wet soil (GRDC, 2010).

141. As noted above, safflower is very resistant to shattering or lodging (Mündel et al., 2004), so seeds are unlikely to be dispersed by wind or via water runoff from irrigation or rainfall prior to harvest. Residual seeds that fall during harvest could be dispersed by water runoff from rainfall or by strong winds.

142. Typical safflower seed losses during harvest are 3-4% (GRDC, 2010) and the viability of these seed may range from 26 to 84% (McPherson et al., 2009b). Most of these seeds would germinate soon after harvest as safflower seeds have very low dormancy (see Chapter 1, Section 4). In a Canadian study, safflower seed did not persist beyond 2 years at the surface or 1 year when buried (McPherson et al., 2009b). It is not expected that the genetic modifications to safflower would affect seed yield, viability or germination. While the fatty acid composition is altered, the total fatty acid content of seeds, and thus their stored energy content, remains constant. Data from trials conducted with the GM events under DIR 121 suggests that seed lost at harvest germinated within the first 2 months post-harvest with no further volunteers observed over the following seven months even though conditions were conducive for germination. GM safflower seeds grown in the greenhouse under DIR 121 were reported to germinate and establish at the same rate as non-GM comparators.

143. Small birds can feed on ripening safflower seed in the head, and cockatoos can chew off safflower plants at the base in order to access the seeds (GRDC, 2010). Safflower seeds that have passed through the digestive systems of several bird species (blackbirds, mallard ducks, pigeons and pheasants) were observed to be no longer viable, but did remain viable in the oesophagus, crop and gizzard regions for several hours (Cummings et al., 2008). This study was on Northern Hemisphere bird species and results may differ for Australian bird species such as galahs, corellas or bush turkeys. The researchers also mentioned birds that hoard or cache seeds such as jays, crows and ravens, as potential transport vectors of safflower seeds. It is not known whether Australian birds carry seeds away for later consumption. Safflower seeds did not attach to the plumage of the birds due to the

smooth nature of safflower seeds, however, seeds were transported externally on soil attached to feet or legs of pigeons and pheasants (Cummings et al., 2008).

144. Large animals are generally deterred from grazing on standing safflower by its spines. Safflower seeds are firmly held within their seed heads, which limits their accessibility to rodents. Residual GM seeds post-harvest may attract animal predation, and could be transported and hoarded by rodents.

Potential harm

145. As discussed in Section 2.3 of this Chapter, all plants have the potential to lead to harm in certain environments. For the purposes of this document, plant species causing significant levels of one or more of these harms are called "weeds". A plant species may be weedy in one or more land uses, such as dryland cropping or nature conservation.

146. As summarised in Chapter 1, Section 6.3, safflower is naturalised throughout Australia, primarily as an agricultural or ruderal weed and is classified as a category 1 weed in agricultural ecosystems or a category 3 weed in natural ecosystems in some States. Anecdotal evidence from safflower farmers (GRDC 2010) and weed risk assessment experts (personal communication, Stephen Johnson 2014) in Australia indicate that safflower is not a significant weed in natural ecosystems. In reference to native habitats, there would have to be large numbers of GM plants before the establishment of native plants was affected.

147. As discussed in Risk scenario 1, the introduced gene products are not expected to be toxic to humans or other organisms and it is unlikely that the GM safflower plants would have higher toxicity and/or allergenicity than non-GM safflower. No phenotypic changes were observed between GM safflower and non-GM safflower grown in greenhouses or in the field under DIR 121. The phenotypic and agronomic performances of the GM safflower lines were assessed in field trials under licence DIR 131, and showed that GM safflower is similar to the non-GM safflower parent in agronomic performance. In the unlikely event of GM safflower plants establishing themselves beyond intended cropping areas, this trait would not be expected to lead to populations of GM safflower that cause any environmental harms associated with weedy plants, such as reduced establishment or yield of desirable plants, or reduced biodiversity.

Conclusion

148. Risk scenario 2 is not identified as a substantive risk due to the introduced gene fragments not coding for any proteins, the biosynthetic pathway they will affect being involved in the production of compounds that have not been associated with toxic or allergenic reactions, and the modified trait not being associated with weediness. 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 gene construct for high oleic acid composition.
Causal pathway	⬇ Commercial cultivation of GM safflower producing seed containing high oleic acid content. ⬇ GM safflower seed consumed by pest animals. ⬇ Increased fitness of pest animals. ⬇ Impact of these animals on native or desirable vegetation. ⬇

Potential harm	Reduced establishment or yield of desirable vegetation. OR Reduced biodiversity.

Risk source

149. The source of potential harm for this postulated risk scenario is the introduced RNAi gene silencing construct.

Causal pathway

150. Safflower seeds are a food source for a range of species including mammals, birds and invertebrates (OGTR, 2018). The applicant proposes that GM safflower with high oleic acid content would be cultivated on a commercial scale. Consequently, pest animals such as rabbits, rodents, bird species or pest insects would be able to access GM safflower in the fields. Other large animals such as kangaroos or wild pigs may also be able to access the GM safflower.

151. As discussed in Risk scenario 2, small birds can feed on ripening safflower seed in the head, and cockatoos can chew off safflower plants at the base in order to access the seeds (GRDC, 2010). Little corellas can cause significant damage to commercial oilseed crops by digging up freshly sown seed, severing plants and eating seed in the head (Tracey et al., 2007). Other bird species that are known to cause damage to oilseed crops include galahs and sulphur-crested cockatoos (Tracey et al., 2007). For some larger animals such as cattle, foraging or grazing is minimal due to the spiny nature of mature safflower plants, but sheep and goats are not irritated by the spines (Cummings et al., 2008). Feral pigs or boars are very destructive and difficult to exclude from fields and native animals may also feed on safflower (GRDC, 2010; OGTR, 2018). In general, however, pests such as pigs, kangaroos and birds are deterred from grazing safflower by its spines and unpalatability (GRDC, 2010). Indeed, the physical traits and unpalatability of safflower deter potential pest species from damaging the crop, making it a low pest maintenance crop (GRDC, 2010).

152. The major potential pest for safflower is likely to be rodents. Safflower seeds are firmly held within their seed heads and are highly shatter resistant, which limits their accessibility to a number of species (OGTR, 2018). However, residual GM seeds present on the soil surface post-harvest may attract animal predation, and could be transported and hoarded by rodents.

153. On broadacre properties, where crops would be the main food source for rodent pests, it is possible that the GM safflower could constitute a significant part of their diet (though it should be noted that safflower is currently a very minor crop in Australia). As discussed in Chapter 1 (Section 5) and Risk scenario 1, the expression of the introduced gene silencing construct is highly unlikely to result in the production of a novel toxin or allergen. Rather, for many species it may have a beneficial role in their growth and general health. Thus it could be posited that availability of additional oleic acid in the diet may potentially lead to an increase in fitness, with a resulting impact on surrounding vegetation.

Potential harm

154. The GM safflower proposed for release has been modified to increase the levels of oleic acid (a monounsaturated FA), with a corresponding decrease in linoleic acid (a polyunsaturated FA) and palmitic acid (a saturated FA)(Wood et al., 2018). As discussed in Chapter 1, Section 5.2.3, increased oleic acid intake has been shown to be associated with a number of health benefits in humans, including lowering cholesterol and blood pressure; and reducing cardiovascular and coronary heart disease (Sales-Campos et al., 2013; Calder, 2015). However, it is unclear if these health benefits will contribute to the reproductive fitness and competitiveness of short-lived pest species such as rodent pests or small birds. A study of pigs fed a diet supplemented by high oleic sunflower oil found no effect on growth parameters and meat quality (Sardi et al., 2010). For birds, a study of Japanese quail hens found that palmitic acid, a saturated fat, played a more important role in reproductive

performance as compared to oleic and linoleic acid (Vilchez et al., 1992). In another study, tree swallow chicks that were fed a diet high in polyunsaturated fats grew faster and had better body condition compared to chicks fed a diet low in polyunsaturated fats (Twining et al., 2016). While it is possible that dietary oleic acid could undergo further desaturation following ingestion by birds (thereby increasing available levels of polyunsaturated fatty acids), oleic acid is already widely available to pest bird species that frequent non-GM high oleic oil crops such as canola. No marked changes in observable fitness have been reported for these birds over time.

155. It is also unclear if safflower provides a nutritious diet to pest species. The use of safflower seed or seed meal as livestock feed is limited due to high fibre content and low protein quality. It is unsuitable for monogastric animals such as swine and poultry, as non-removal of hulls results in a high fibre content (30–40%) (Dajue and Mündel, 1996). The high fibre content also presents palatability and digestibility problems for ruminants. Compared to other oilseed meal, the quality of safflower protein is low due to its deficiency in lysine, methionine and isoleucine, the sulphur containing amino acids. Additionally, the protein fraction of the meal contains two phenolic glucosides, the bitter-flavoured matairesinol-β-glucoside and the purgative 2-hydroxyarctiin-β-glucoside (Heuzé et al., 2012).

156. If consumption of seed from high oleic acid safflower were to enhance the fitness of pest animals, this could lead to a greater impact of these animals on native or desirable vegetation or increased competition for desirable animals/birds. It should be noted in this context, however, that there have been no reports of increased pest activities in areas where non-GM high oleic safflower varieties are grown. As discussed above, bird species are known to damage oilseed crops, including safflower (GRDC, 2011). Bird control measures include not growing safflower near forested areas, harvesting as soon as possible and using other crops as alternative food sources (Tracey et al., 2007; GRDC, 2011).

157. In the case of broadacre crops, rodents are subjected to control measures including maintaining crop hygiene to reduce rodent numbers, monitoring rodent activity and baiting. These are used routinely to minimise crop loss from pests (GRDC, 2011) and would help to reduce the chance for rodent populations to access the GM safflower crops. Nonetheless, control measures are not fully effective in the event of mouse plagues, which are seen to occur in years of good rainfall, moist soils and abundant food. In particular, an increase in mouse plagues in recent years has been attributed to changes in agricultural practice that sees growing of two summer crops under irrigation with a following winter cereal crop (NSW DPI, 2018). Thus, overall abundance of available food is more likely to influence potential problems posed by an ingesting rodent pest species than the seed oil profile of a minor crop.

158. If pest insects consuming high oleic acid safflower became stronger and more competitive because of the health benefits of high oleic acid diets (Chapter 1, Section 5.2.3), they may cause more damage to other crops in agriculture areas, or reduce native or desirable vegetation and compete with desirable insect species. However, pest insects in safflower fields are readily controlled by current pest management practices, including the application of various insecticides (GRDC, 2010). Therefore, the chance for pest insects to access increased oleic acid in the GM safflower seed is low.

Conclusion

159. Risk scenario 3 is not identified as a substantive risk because exposure of pest animals and insects to increased oleic acid in the GM safflower seed is low, other agricultural factors are more likely to influence pest numbers and fitness, and pests are controlled by pest management practices in the safflower fields. 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 gene construct for high oleic acid composition.
Causal pathway	⬇ Commercial cultivation of GM safflower expressing the introduced genes. ⬇ Dispersal of GM safflower seed outside intended cropping areas. ⬇ Vertical transfer and expression of introduced genes in other sexually compatible plants ⬇ Establishment of populations of volunteer GM plants.
Potential harm	Increased toxicity or allergenicity in people or toxicity to other organisms. OR Reduced establishment or yield of desirable vegetation. OR Reduced biodiversity.

Risk source

160. The source of potential harm for this postulated risk scenario is the introduced RNAi gene silencing construct.

Causal pathway

161. Pollen from GM safflower lines could be transferred outside the cropping areas and fertilise sexually compatible plants, whether they be non-GM safflower or plants from another sexually compatible species.

162. Vertical gene flow is the transfer of genetic information from an individual organism to its progeny by conventional heredity mechanisms, both asexual and sexual. In flowering plants, pollen dispersal is the main mode of gene flow (Waines and Hegde, 2003). For GM crops, vertical gene flow could therefore occur via successful cross-pollination between the crop and neighbouring crops, plants, related weeds or native plants (Glover, 2002). Alternatively, if seed was dispersed outside the cropping areas, GM plants may grow and subsequently disperse pollen. Hybrid plants possessing the introduced genes may form the basis for the spread of these genes in other varieties of safflower, or other sexually compatible plant species. It should be noted that vertical gene flow *per se* is not considered an adverse outcome, but may be a link in a chain of events that may lead to an adverse outcome. Baseline information on vertical gene transfer associated with non-GM safflower plants can be found in *The Biology of* Carthamus tinctorius *L. (safflower)* (OGTR, 2018).

163. Safflower is predominantly self-pollinating, with cross-pollination rates averaging 10% (USDA-APHIS, 2008). Safflower pollen is large and sticky, with most pollen grains transported by wind less than 1 m from the parent plant (Claassen, 1950). Thus, wind plays a minor role in safflower cross-pollination. Insects are considered the main method for cross-pollination, with honeybees the predominant insect pollinator in mainland Australia (OGTR, 2018). Field trials investigating gene flow in India (Kadam and Patankar, 1942), the United States (Claassen, 1950), Germany (Rudolphi et al., 2008), Canada and Chile (McPherson et al., 2009a), Spain (Velasco et al., 2012) or Morocco (Nabloussi et al., 2013) have shown that cross-pollination frequencies and distances vary with cultivars and external factors such as climate or presence of pollinators (see *The Biology of* Carthamus tinctorius *L. (safflower)* (OGTR, 2018) for review).

164. Honeybees foraging at long distance have been documented in safflower (Gary et al., 1977). However, field-to-field pollination mediated by honeybees has been estimated as low: a maximum gene flow of 0.005-0.05% between fields was calculated by Cresswell (2010) using mathematical

models. The author describes honeybees as often showing fidelity to a particular feeding site, thus limiting field-to-field gene flow.

165. Pollen transport is not the only barrier to cross-pollination: once dispersed, pollen grains need to compete with the floret's own pollen to result in outcrossing. Flower anatomy of safflower promotes self-pollination: the emerging stigma is in close contact with the anthers and may be covered in pollen by the time it is fully expanded (Claassen, 1950; Cresswell, 2010). Moreover, pollen carryover is low, with transported safflower pollen reported to only be moved to the next visited floret (Cresswell et al., 2002).

166. As discussed in Chapter 1, there are four weedy *Carthamus* species that may be present in Australia: *C. lanatus*, *C. leucocaulos*, *C. dentatus* and *C. glaucus*. There are no reports of any of these weeds crossing with safflower under natural conditions. Artificial crosses have resulted in the production of hybrids that are either sterile or showing low fertility (OGTR, 2018). GM safflower could theoretically cross with these species at very low rate but if these crosses were to produce any offspring, such hybrids are highly likely to be sterile (McPherson et al., 2004; Mayerhofer et al., 2011). Furthermore, *C. lanatus* and *C. leucocaulos* being classified as noxious weeds in Australia are under active control at the State level. For example, *C. leucocaulos* is part of an eradication program at the State level in WA (Groves et al., 2003). Therefore, these two species are unlikely to be allowed to flower in cropping areas.

Potential harm

167. If pollen from GM safflower lines was to be dispersed, resulting hybrid plants could spread and persist in the environment, leading to increased toxic reactions or allergenicity in people and/or other desirable organisms. Hybrids expressing the introduced RNAi gene silencing construct could also reduce the establishment and yield of desired plants and subsequently reduce biodiversity.

168. The increased oil content trait that has been introduced into the GM plants of this application could combine, via vertical gene transfer, with traits of other non-GM commercially cultivated safflower plants, or other related species. However, there is no reason to believe that the resulting plants would possess a level of toxicity or allergenicity greater than that of either parent, or a level of weediness greater than that of either parent.

169. As discussed in Risk scenario 1, the introduced RNAi gene silencing construct itself is not expected to be toxic to humans or other organisms, nor is the oleic acid product expected to be toxic or allergenic. Properties of the RNAi gene silencing construct and its impact on oil production in the seed are not expected to differ in a hybrid background. Therefore, in the rare event of the vertical transfer from the GM safflower lines to non-GM safflower plants or sexually compatible species, it is expected that the introduced RNAi gene silencing construct in the subsequent hybrid will have the same properties as in the GM safflower parent.

170. As discussed in Risk scenario 2, the introduced RNAi gene silencing construct is unlikely to make the GM safflower lines weedier and (as above) this is not expected to change in a hybrid background resulting from cross-pollination.

Conclusion

171. Risk scenario 4 is not identified as a substantive risk due to the limited ability of safflower pollen to be dispersed at long distances, the limited ability to cross with weedy related species, and the expected lack of toxicity, allergenicity or increased weediness in any offspring of the GM plants and other sexually compatible plants. Therefore this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

172. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis[10]. There are several types of uncertainty in risk analysis (Clark and Brinkley, 2001; Hayes, 2004; Bammer and Smithson, 2008). These include:

- uncertainty about facts:
  - knowledge – data gaps, errors, small sample size, use of surrogate data
  - variability – inherent fluctuations or differences over time, space or group, associated with diversity and heterogeneity
- uncertainty about ideas:
  - description – expression of ideas with symbols, language or models can be subject to vagueness, ambiguity, context dependence, indeterminacy or under-specificity
  - perception – processing and interpreting risk is shaped by our mental processes and social/cultural circumstances, which vary between individuals and over time.

173. Uncertainty is addressed by approaches including 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.

174. Uncertainty can arise from a lack of experience with the GMO.

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

176. Post release review (PRR) will be used to address uncertainty regarding future changes to knowledge about the GMO or the receiving environment (Chapter 3, Section 4). PRR is typically required for commercial releases of GMOs, which generally do not have limited duration.

Section 4 Risk evaluation

177. 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.

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

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

179. Four risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. The level of risk for each scenario was considered negligible in relation to

[10] A more detailed discussion of uncertainty is contained in the Regulator's *Risk Analysis Framework* available from the OGTR website or via Free call 1800 181 030.

both the seriousness and likelihood of harm, and by considering both the short and long term. The principal reasons for these conclusions are summarised in Table 6.

180. The *Risk Analysis Framework* (OGTR, 2013), which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no controls are required to treat these negligible risks. The Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment[11].

[11] As none of the proposed dealings are considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator has allowed up to 8 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

Chapter 3 Risk management plan

Section 1 Background

181. 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 proposed licence conditions.

182. 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.

183. 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 are also required to be reported to the Regulator.

184. 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

185. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed release of GM safflower. These risk scenarios were considered in the context of the large scale of the proposed release and the receiving environment. The risk evaluation concluded that no containment measures are required to treat these negligible risks.

Section 3 General risk management

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
- testing methodology
- identification of the persons or classes of persons covered by the licence
- reporting structures
- access for the purpose of monitoring for compliance.

3.1 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 Go Resources Pty Ltd suitable to hold a licence. The licence includes a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability.

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

3.2 Testing methodology

190. Go Resources Pty Ltd is required to provide a method to the Regulator for the reliable detection of the GMO, and the presence of the introduced genetic materials in a recipient organism. This instrument would be required prior to conducting any dealings with the GMO.

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

191. Any person, including the licence holder, may conduct any permitted dealing with the GMO.

3.4 Reporting requirements

192. 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 release.

193. The licence holder is also obliged to submit an Annual Report containing any information required by the licence.

194. There are also provisions that enable the Regulator to obtain information from the licence holder relating to the progress of the commercial release (see Section 4, below).

3.5 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 the Regulator, or a person authorised by the Regulator, to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

196. 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 Post release review

197. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator takes account of the likelihood and impact of an adverse outcome over the foreseeable future, and does not disregard a risk on the basis that an adverse outcome might only occur in the longer term. However, as with any predictive process, accuracy is often greater in the shorter rather than longer term.

198. The Regulator has incorporated a requirement in the licence for ongoing oversight to provide feedback on the findings of the RARMP and ensure the outcomes remain valid for future findings or changes in circumstances. If a licence was issued, this ongoing oversight would be achieved through post release review (PRR) activities. The three components of PRR are:

- adverse effects reporting system (Section 4.1)
- requirement to monitor specific indicators of harm (Section 4.2)
- review of the RARMP (Section 4.3).

The outcomes of these PRR activities may result in no change to the licence or could result in the variation, cancellation or suspension of the licence.

4.1 Adverse effects reporting system

199. Any member of the public can report adverse experiences/effects resulting from an intentional release of a GMO to the OGTR through the Free-call number (1800 181 030), mail (MDP 54 – GPO Box 9848, Canberra ACT 2601) or via email to the OGTR inbox (ogtr@health.gov.au). Reports can be made at any time on any DIR licence. Credible information would form the basis of further investigation and may be used to inform a review of a RARMP (see Section 4.3 below) as well as the risk assessment of future applications involving similar GMOs.

4.2 Requirement to monitor specific indicators of harm

200. Collection of additional specific information on an intentional release provides a mechanism for 'closing the loop' in the risk analysis process and for verifying findings of the RARMP, by monitoring the specific indicators of harm that have been identified in the risk assessment.

201. The term 'specific indicators of harm' does not mean that it is expected that harm would necessarily occur if a licence was issued. Instead, it refers to measurement endpoints which are expected to change should the authorised dealings result in harm. Should a licence be issued, the licence holder would be required to monitor these specific indicators of harm as mandated by the licence.

202. The triggers for this component of PRR may include risk estimates greater than negligible or significant uncertainty in the risk assessment.

203. The characterisation of the risk scenarios discussed in Chapter 2 did not identify any risks greater than negligible. Therefore, they were not considered substantive risks that warranted further detailed assessment. Uncertainty is considered to be low. No specific indicators of harm have been identified in this RARMP for application DIR 158. However, specific indicators of harm may also be identified during later stages, e.g. following the consideration of comments received on the consultation version of the RARMP, or if a licence were issued, through either of the other components of PRR.

204. Conditions have been included in the licence to allow the Regulator to request further information from the licence holder about any matter to do with the progress of the release, including research to verify predictions of the risk assessment.

4.3 Review of the RARMP

205. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would take into account any relevant new information, including any changes in the context of the release, to determine if the findings of the RARMP remained current. The timing of the review would be determined on a case-by-case basis and may be triggered by findings from either of the other components of PRR or be undertaken after the authorised dealings have been conducted for some time. If the review findings justified either an increase or decrease in the initial risk estimate(s), or identified new risks to people or to the environment that require management, this could lead to changes to the risk management plan and licence conditions.

Section 5 Conclusions of the consultation RARMP

206. The risk assessment concludes that the proposed commercial release of GM safflower poses negligible risks to the health and safety of people or the environment as a result of gene technology and that these negligible risks do not require specific risk treatment measures.

207. However, general conditions have been imposed to ensure that there is ongoing oversight of the release.

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Appendix A: Summary of submissions from prescribed experts, agencies and authorities

The Regulator received several submissions from prescribed experts, agencies[12] and authorities on matters relevant to preparation of the RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. These issues, and where they are addressed in the consultation RARMP, are summarised below.

Submission	Summary of issues raised	Comment
1	Agrees with the issues identified by OGTR for consideration in the RARMP. The RARMP should consider possible benefits for pest species consuming GM safflower or safflower meal.	Noted. The potential for pest species to gain an advantage from consumption of the GM safflower has been addressed in Risk Scenario 3.
2	Noted that the Town does not have safflower growing areas nor have an official policy regarding GM products. Recommends that growing of GM safflower should be undertaken in a way that is safe to both the public and the environment.	Noted. Risks to public safety and the environment from the proposed release are evaluated in Chapter 2, Sections 2.4.1 to 2.4.4 (Risk Scenarios 1 – 4). The consultation RARMP concludes that the risks are negligible.
3	Noted that the information available on the OGTR website regarding horizontal gene transfer is quite dated and could benefit from a review.	An updated version of the reference document was uploaded on the OGTR website on 6 December 2017.
4	Reports that application has negligible risk to health and safety of people and the environment. Specifically notes that the proposed modification to safflower is highly unlikely to increase the species' weed risk. General questions to consider in the RARMP include: • Are the modified traits in the lines approved under DIR 121 and DIR 131 related to those in the current application? • Did the performance of the GM safflowers in DIR 121 and DIR 131 meet expectations in the field trials?	Noted. The relationships between GM safflower lines characterised under licence DIR 121 and 131 and the GMOs in the current application are explained in Chapter 1, Section 5 of the consultation RARMP. Agronomic information that is relevant to the assessment of risks to people and the environment for the GM safflower plants characterised under licence DIR 121 and 131 is described in Chapter 1, Section 5 of the consultation RARMP.
5	Recommends that the RARMP addresses issues around toxicity caused by the use of an RNAi construct including increase in an existing metabolite to a toxic level (either due to suppression of the target or a non-target gene), uptake into herbivorous insects or humans and mammals and off-target silencing in those organisms.	The potential for the introduced gene sequences to produce a toxic product is discussed in Chapter 1, Section 5.2.2. The potential for off-target effects is discussed in

[12] Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian government agencies and the Minister for the Environment.

Submission	Summary of issues raised	Comment
	The toxicity of the selectable marker gene (*hph*) protein product should also be reveiwed in this RARMP.	Chapter 2, Risk scenario 1. Potential toxicity resulting from the introduced *hph* gene is discussed in Chapter 1, Section 5.2.2 and Chapter 2, Section 2.1.
	C. tinctorius is not recorded in the Australian government's 'Weeds of National Significance' list, the 'National Environment Alert List', or the 'Noxious Weed List for Australian States and Territories'. It can be a minor problem in both agricultural systems, and natural ecosystems. It is recommended that the RARMP thoroughly cover both the factors that restrict the ability of safflower to spread and persist in natural ecosystems, and the potential for the genetic modification to increase the ability of the GM plants to spread and persist.	These issues are discussed in Chapter 1, Sections 6.2 and 6.3; Chapter 2, Risk scenarios 2 and 4.
	It is noted that there are 4 species of *Carthamus* in Australia. The likelihood for gene flow, and the potential adverse effects to the environment of the introgression of the high oleic acid composition trait into any other species, should be covered in the RARMP.	The possibility of gene transfer and the potential adverse effects to the environment are addressed in Chapter 1 Section 6.3 and Chapter 2, Risk Scenario 4.
	There is extensive experience in the general management of safflower in agricultural settings. This experience should be directly applicable to the management of the GM plants in this application, and therefore it is recommended that it is discussed in the RARMP.	General management of the GM safflower in agricultural settings is discussed in Chapter 1, Section 6.1.
6	Notes that the application is for GM safflower and not canola, so expects that prior to approval of the commercial release of GM safflower, the AOF will prepare a set of guidelines and standards for GM safflower considering the GO Resources 'closed-loop preserved (CLIP)' quality assured management system. Some members believe that the statement in Part 12: Question 12.1(b) that 'Australia is the only country that currently classifies safflower as a weed (Groves et al. 2003)' is not accurate. Suggests the statement should be revised.	Noted. The weed risk potential of safflower outside of cultivation is discussed in Chapter 1, Section 4 of the consultation RARMP. Weediness of the GM safflower is evaluated in Chapter 2, Section 2.4.4 Risk Scenario 4.

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

The Regulator received a number of submissions from prescribed experts, agencies and authorities on the consultation RARMP. All issues raised in submissions that related 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	No comment provided, as does not have a specialist scientific expert to make an assessment.	Noted.
2	Recognises that the release will be beneficial to the agricultural sector and therefore supports the commercial release of GM safflower.	Noted.
3	Notes Section 4 of South Australia's *Genetically Modified Crops Management Regulations 2008* states that 'the whole of the State is designated as an area in which no genetically modified crops may be cultivated'.	State and territory moratoria on the growing of GM crops relate to issues of marketability, agricultural trade and segregation. They do not relate to protection or safety of human health or the environment, and are a matter for state and territory governments, not the Regulator. The licence for DIR 158 does not authorise dealings with GM safflower that are otherwise prohibited as a result of the operation of State or Territory legislation.
	Following a public consultation, adopted a 'Genetically Modified Crops' policy on 25 September 2012 whereby they do not support the growing of genetically modified crops within its district. Determined that there is an absence of credible and independent scientific evidence that GM crops are safe for people or the environment. Policy will remain in effect until such evidence can be presented.	The RARMP prepared for each application for a licence to grow a GM crop includes a thorough and critical assessment of data supplied by the applicant, together with a comprehensive review of other relevant national and international scientific literature. Scientific and other literature is monitored for any new information relevant to GMOs and GM foods, and assessed for its potential to impact on the health and safety of people and the environment.
	Notes that the RARMP concludes that 'risks to the health and safety of people or the environment from the proposed dealings are negligible', and that these risks can be 'managed so as to protect people and the environment'.	Noted.
	Urges OGTR to withhold permission for the commercial use of any GM crops until their safety has been credibly and independently demonstrated, rather than their risk deemed negligible.	The Regulator's approach to risk analysis can be found in the Risk Analysis Framework at the OGTR website. The RARMP is based on the best available scientific evidence, further informed by input from experts, agencies and the Gene Technology Technical Advisory Committee.
4	Did not identify any concerns relating to this GM safflower intended for industrial use. Noted that the oil is intended for industrial use, but commented that the industrial product could	Noted. As discussed in Chapter 1, Section 5 of the RARMP, FSANZ has previously approved oil from

Submission	Summary of issues raised	Comment
	make its way to the food chain so FSANZ's concurrent assessment of the intended food product is of interest.	GM high oleic soybean as safe for human consumption. The applicant has submitted an application to FSANZ to amend the Food Standards Code to include the GM safflower.
5	Agreed that given oleic acid is present broadly in the environment, an increase in content in the GMO would not pose any additional risks to human health or the environment. Agrees with the overall conclusions of the RARMP.	Noted.
6	Notes that applicant has also submitted an Application (A1156) to FSANZ seeking approval for food derived from the same GM safflower lines as those assessed in the RARMP. A decision on the FSANZ application is due by late October 2018. No specific comments on the RARMP.	Noted.
7	Agrees with the overall conclusions of the RARMP. Recommends that the RARMP includes further discussion on the risk of increased fitness of pest birds due to consumption of GM safflower seeds. The RARMP should include further discussion of exposure of pest birds to safflower seeds and discuss the extent that risk management practices may control bird pests. The RARMP would benefit from further discussion on the impact of fatty acids on the fitness of pest species. Notes reports that certain fatty acids may increase reproductive ability in pigs, insects and swallows.	Noted. Information has been added to Chapter 2, Section 2.4.3 Risk Scenario 3 where appropriate. There are already abundantly available seed sources of oleic acid in the agricultural environment and additional ingestion of high oleic safflower is unlikely to increase reproductive fitness of pest birds. Information has been added to Chapter 2, Section 2.4.3 Risk Scenario 3 where appropriate. Risk scenario 3 concluded that potential harm to the environment through increased fitness of pest birds was negligible. Therefore, control of bird damage is a matter affecting agricultural productivity, a consideration that is outside the scope of the Regulator's assessment. Information has been added to Chapter 2, Section 2.4.3 Risk Scenario 3 where appropriate. The reports relate to both monounsaturated and polyunsaturated fatty acids. They are referenced where relevant to a high oleic acid diet.
8	Application has negligible risks to the health and safety of people and the environment. Noted that the proposed modification to safflower is highly unlikely to increase the species' weed risk.	Noted.
9	Concerned regarding the propensity for weediness of safflower species in agricultural areas, as highlighted in the OGTR's document '*The Biology of* Carthamus tinctorius *L. (safflower)*. Overall, supported the OGTR's conclusion that	The weed risk potential of safflower outside of cultivation is discussed in Chapter 1, Section 4 of the RARMP. The potential for the genetic modification to increase weediness of the GM safflower (relative to non-GM safflower) is evaluated in Chapter 2, Section 2.4.4 Risk Scenario 4 and the risk was found to be negligible. Noted.

Submission	Summary of issues raised	Comment
	the proposed dealing poses negligible risk to human health and safety and the environment.	

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

The Regulator received one submission from the public on the consultation RARMP. The issues raised in this submission is 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	Concerned that the experimenting with crops to have a higher tolerance to herbicides will lead to growers needing to use stronger herbicides as weeds become herbicide resistant. States that this is already happening in the South West of Western Australia, where farmers are forced to use stronger chemicals as the weeds mutate to withstand the herbicides and cannot be allowed to continue.	The safflower has been genetically modified to alter oleic acid composition. Herbicide tolerance has not been altered. Issues relating to herbicide use are outside the scope of the Regulator's assessments. The APVMA has regulatory responsibility for agricultural chemicals, including pesticides and herbicides, in Australia.