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

June 2025

Risk Assessment and Risk Management Plan for

DIR 211

Limited and controlled release of safflower genetically modified for dairy protein production and altered fat composition

Applicant: Miruku Australia Pty Ltd

Summary of the Risk Assessment and Risk Management Plan for Licence Application No. DIR 211

Decision

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

The application

Applicant	Miruku Australia Pty Ltd (Miruku)
Project title	Limited and controlled release of safflower genetically modified for dairy protein production and altered fat composition[1]
Parent organism	Safflower (*Carthamus tinctorius* L.)
Introduced genes[2]	Introduced genes producing dairy protein and altering fat composition: • modified β-casein gene based on the gene from *Bos taurus* (cattle) for dairy protein production • RNA hairpin constructs to down-regulate endogenous fatty acid genes *FAD2* and *SAD*. Introduced marker genes: • *bar* gene from bacterium *Streptomyces hygroscopicus* for tolerance to the herbicide glufosinate • *hph* gene from *Streptomyces hygroscopicus* for hygromycin antibiotic resistance • codon-optimised *gusA* gene from *Staphylococcus* sp. for visual marker selection.
Genetic modification method	*Agrobacterium*-mediated transformation
Number of lines	Up to 120 lines
Previous releases	None in Australia or overseas
Proposed locations	Up to 52 sites to be selected from 135 possible local government areas in New South Wales, Victoria, Western Australia and South Australia

[1] The title of the project as supplied by the applicant is "Limited and controlled release of Safflower genetically modified for dairy protein and fat composition".

[2] Confidential Commercial Information: Some details about the introduced genetic elements in GM safflower have been declared as Confidential Commercial Information under section 185 of the Act. This information was provided to the prescribed experts and agencies that were consulted on this application. CCI is not available to the public.

Proposed release size	Up to 1 ha in 2025, 5 ha in 2026, 50 ha in 2027, 225 ha in 2028, and 700 ha in 2029, totalling a maximum of 981 ha over the period of release
Proposed period of release	From issue of licence until December 2029
Principal purpose	To produce dairy protein and alter fat composition in GM safflower under field conditions

Risk assessment

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

Credible pathways to potential harm that were considered included exposure of people or other desirable organisms[3] to the GM plant material, potential for persistence or dispersal of the GMOs, and transfer of the introduced genetic material to non-GM safflower plants. Potential harms associated with these pathways included toxicity and allergenicity to people, toxicity to desirable animals, and environmental harms due to weediness.

The risk assessment concludes that risks to the health and safety of people or the environment from the proposed dealings are negligible. No specific risk treatment measures are required to manage these negligible risks. The principal reasons for the conclusion of negligible risks are that the proposed limits and controls, such as not using GM plant material in commercial human food or animal feed, will effectively minimise exposure to the GMOs. In addition, there is no evidence to suggest the introduced genetic modifications would lead to harm to people or the environment.

Risk management

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions.

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

[3] Desirable organisms are those that are valued and should be protected, while undesirable organisms cause harm and should be controlled (OGTR, 2013). This is determined by legislation, government policies, national and international guidance material, and widely acceptable community norms. Undesirable plants that cause economic, social or environmental harm, or harm to human/animal health, are called weeds. Animals that cause harm are known as pests.

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Abbreviations

AICIS	Australian Industrial Chemicals Introduction Scheme
APVMA	Australian Pesticides and Veterinary Medicines Authority
CCI	Confidential Commercial Information
CFIA	Canadian Food Inspection Agency
CMA	Cow's milk allergy
Da	Daltons
DAFF	Department of Agriculture, Fisheries and Forestry
dsRNA	Double-stranded RNA
DIR	Dealings involving Intentional Release
FSANZ	Food Standards Australia New Zealand
GM(O)	Genetically modified (organism)
GUS	β-glucuronidase
ha	Hectare(s)
HGT	Horizontal gene transfer
IBC	Institutional Biosafety Committee
kDa	Kilodaltons
LGA	Local Government Area
m	Metre(s)
miRNA	micro RNA
NLRD	Notifiable Low Risks Dealings
NSW	New South Wales
OGTR	Office of the Gene Technology Regulator
PAT	Phosphinothricin acetyltransferase
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
RNAi	Ribonucleic acid interference
SA	South Australia
sRNA	Small RNA
siRNA	Short interfering RNA
TGA	Therapeutic Goods Administration
the Act	The *Gene Technology Act 2000*
UTR	Untranslated region
Vic	Victoria
WA	Western Australia

Chapter 1 Risk assessment context

Section 1 Background

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

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

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

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

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

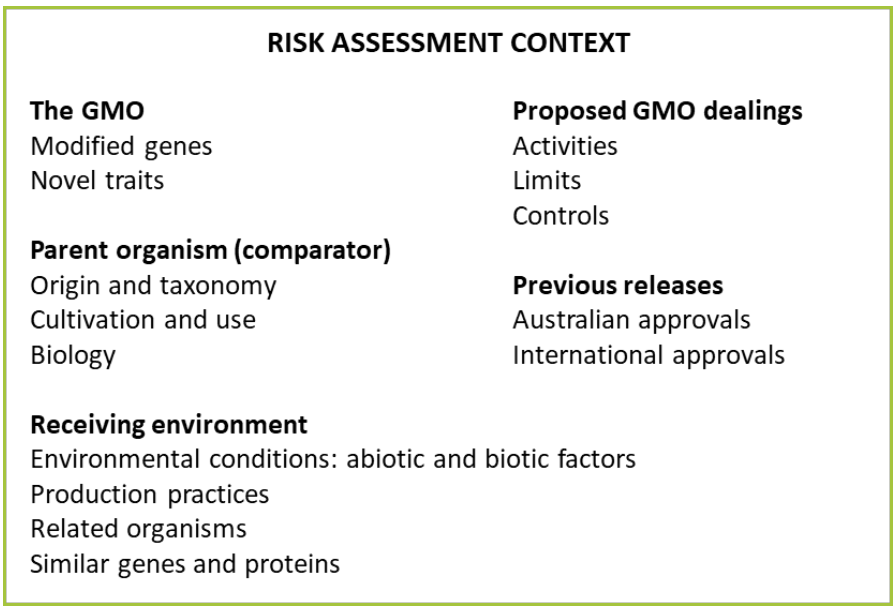

Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR, and the Risk Analysis Framework

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

7. Section 52 of the Act requires the Regulator to seek comment on the RARMP from agencies - the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, Australian local councils and the Minister for the Environment - and from the public. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. No public submissions were received.

1.1 Interface with other regulatory schemes

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. The GMOs and any proposed dealings may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration (TGA), the Australian Industrial Chemicals Introduction Scheme (AICIS) and the Department of Agriculture, Fisheries and Forestry (DAFF). These dealings may also be subject to the operation of State legislation recognising an area as designated for the purpose of preserving the identity of GM crops, non-GM crops, or both GM crops and non-GM crops, for marketing purposes.

9. To avoid duplication of regulatory oversight, risks that have been considered by other regulatory agencies will not be re-assessed by the Regulator.

Section 2 The proposed dealings

10. Miruku Australia Pty Ltd (Miruku, the applicant) proposes to release multiple GM safflower lines into the environment under limited and controlled conditions. The GM plants have been genetically modified for dairy protein production and altered fat composition.

11. The purpose of the release is to assess dairy protein production and altered fat composition in GM safflower under field conditions. The applicant will also evaluate agronomic performance of the GM safflower lines in the field.

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

- conduct experiments with the GMOs
- breed the GMOs
- propagate the GMOs
- use the GMOs in the course of manufacture of a thing that is not the GMOs
- grow the GMOs
- transport the GMOs
- dispose of the GMOs

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

13. Initial transformation of the GMOs will occur in Australia under an Notifiable Low Risk Dealing (NLRD).

14. GM plant material would not be used for commercial human food or animal feed.

15. The GM seeds will be processed to release the protein-fat components to use in food products that may only be used in human sensory testing to assess their feel, smell, taste and appearance. They will not be used for commercial food or feed. Sensory testing would result in negligible consumption of the components from the GM seeds as the products are not intended to be swallowed during testing. These trials would only occur if Miruku obtains the appropriate approvals for each trial in accordance with the National Statement on Ethical Conduct in Human Research.

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

16. The release is proposed to take place between May 2025 and December 2029. Planting would occur primarily during the winter cropping season, but occasionally a summer crop cycle may also be used.

17. GM safflower is proposed to be grown at up to 52 trial sites over the period of release. The proposed maximum number of sites, planting area per site, combined total planting area for each year, and cumulative maximum total planting area are detailed Table 1.

Table 1. Proposed duration and maximum number of sites and planting area per year

Year	Maximum number of sites per year	Maximum area (ha) per site	Maximum combined area (ha) per year	Cumulative maximum total area (ha)
2025	2	0.5	1	1
2026	5	1	5	6
2027	10	5	50	56
2028	15	15	225	281
2029	20	35	700	981

18. Sites for trial release would be selected from 135 possible local government areas (LGAs) in New South Wales (NSW), Victoria (Vic), Western Australia (WA) and South Australia (SA) (Table 2). The field trials would occur on research stations or private land in rural areas where persons other than those conducting dealings would not have access to the field trial sites.

Table 2. LGAs where GM safflower trial sites may be located

New South Wales	Victoria	Western Australia	South Australia
Berrigan	Ararat	Albany	Adelaide Plains
Bland	Ballarat	Beverley	Barossa
Blayney	Benalla	Boddington	Light
Cabonne	Buloke	Boyup Brook	Wakefield
Coolamon	Campaspe	Bridgetown-Greenbushes	
Coonamble	Central Goldfields	Brookton	
Cootamundra-Gundagai	Colac Otway	Broomehill-Tambellup	
Cowra	Corangamite	Carnamah	
Dubbo	Gannawarra	Coorow	
Edward River	Glenelg	Corrigin	
Federation	Golden Plains	Cranbrook	
Forbes	Greater Bendigo	Cuballing	
Gilgandra	Greater Geelong	Cunderdin	
Greater Hume	Greater Shepparton	Dalwallinu	
Griffith	Hepburn	Denmark	
Gunnedah	Hindmarsh	Donnybrook-Balingup	
Gwydir	Horsham	Dowerin	
Hay	Indigo	Dumbleyung	
Hilltops	Loddon	Esperance	
Inverell	Macedon Ranges	Gnowangerup	
Junee	Mildura	Goomalling	
Leeton	Mitchell	Greater Geraldton	
Liverpool Plains	Moira	Jerramungup	
Lockhart	Moorabool	Katanning	
Mid-Western	Mount Alexander	Kent	
Moree Plains	Moyne	Kojonup	
Murray River	Northern Grampians	Manjimup	
Murrumbidgee	Pyrenees	Merredin	
Muswellbrook	Southern Grampians	Mingenew	

New South Wales	Victoria	Western Australia	South Australia
Narrabri	Strathbogie	Moora	
Narrandera	Swan Hill	Morawa	
Narromine	Wangaratta	Nannup	
Orange	West Wimmera	Narrogin	
Parkes	Wodonga	Northam	
Snowy Valleys	Wyndham	Perenjori	
Tamworth	Yarriambiack	Pingelly	
Temora		Plantagenet	
Upper Hunter		Quairading	
Wagga Wagga		Ravensthorpe	
Walgett		Tammim	
Warren		Three Springs	
Warrumbungle		Toodyay	
Weddin		Victoria Plains	
		Wagin	
		Wandering	
		West Arthur	
		Wickepin	
		Williams	
		Wongan-Ballidu	
		Woodanilling	
		Wyalkatchem	
		York	

19. Only trained and authorised persons would be permitted to deal with the GM safflower.

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

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

- locating each trial site at least 50 m away from the nearest natural waterway
- surrounding each planting area with a 10 m monitoring zone and a 50 m inspection zone that are monitored from 14 days prior to flowering until the entire planting area has completed flowering, to identify and destroy volunteer safflower or related species (See Figure 2)
- surrounding each inspection zone with a 140 m isolation zone where no safflower or sexually compatible species are grown (See Figure 2)
- treating non-GM safflower plants grown on the trial sites as if they are GMOs
- cleaning equipment and clothing after use on trial sites
- bagging or tenting GM safflower
- using particular seeding or harvesting methods or equipment to minimise dispersal of GM plant material
- controlling rodents on trial sites
- restricting access to trial sites to authorised persons, and using fencing to control access by large animals and vehicles
- cleaning of planting areas post-harvest
- tilling and irrigating each planting area during the post-harvest monitoring period
- post-harvest monitoring of the trial sites for 24 months to identify any volunteer safflower, and

destroy volunteers before they reach flowering

- destroying all GMOs not required for further experimentation
- transporting and storing GMOs in accordance with the current Regulator's Guidelines for the Transport, Storage and Disposal of GMOs
- not allowing the GMOs or GM products to be used for commercial human food or animal feed.

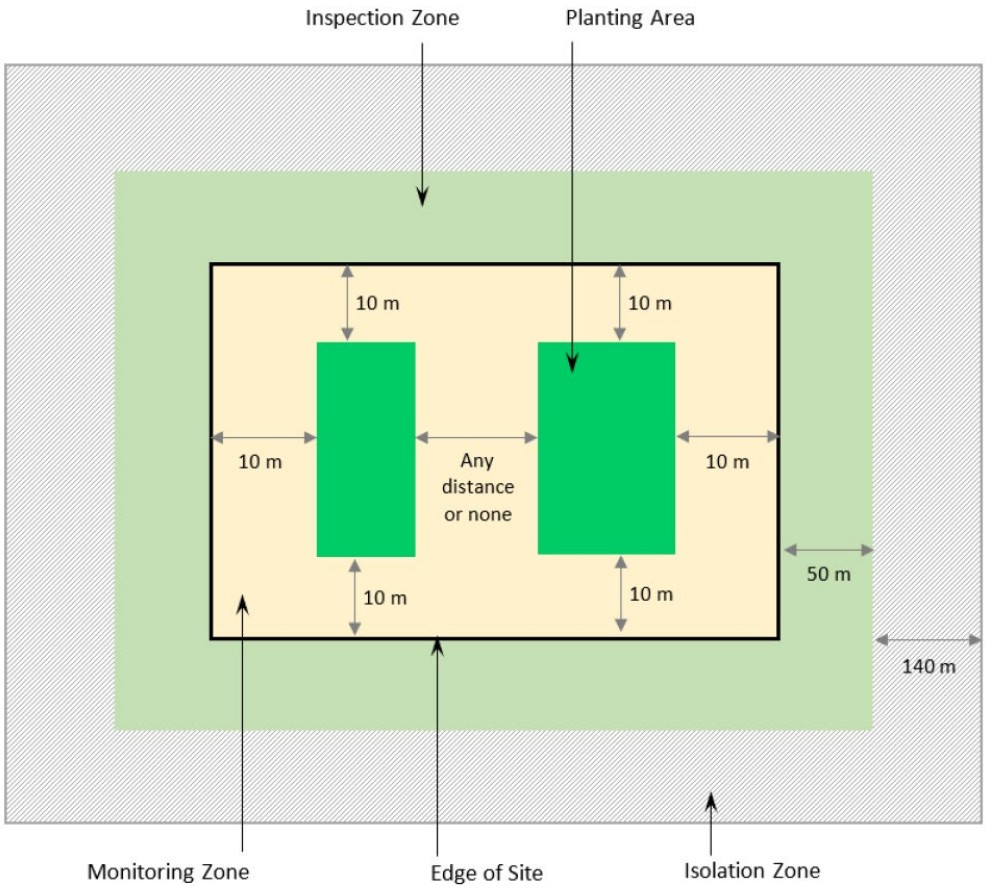

Figure 2. Schematic diagram (not to scale) of proposed trial layout. Each trial site may include one or multiple planting areas.

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

Section 3 The parent organism

22. The parent organism of the GMOs is safflower (*Carthamus tinctorius* L.). Safflower is exotic to Australia.

23. Detailed information about the parent organism is contained in the reference document produced to inform the risk analysis process for licence applications involving GM safflower: *The Biology of* Carthamus tinctorius *L. (safflower)* (OGTR, 2019). This document is available from the Resources page on the OGTR website. Baseline information from this document will be used and referred to throughout the RARMP.

24. Safflower is a minor oilseed crop in Australia, accounting for less than 0.05% of the total cropping area in Australia (ABARES, 2024). Over the past decade, the national annual safflower planting area has ranged from approximately 7,000 to 15,000 ha (ABARES, 2024). Safflower is primarily grown in Australia to extract oil from seeds for use in the food industry, but whole safflower seeds are also produced for use as birdseed (GRDC, 2017). The by-product of oil extraction from safflower seeds is used as meal for stockfeed. Cultivars of safflower are comprised primarily of varieties producing seed high in oleic acid and varieties

high in linoleic acid, with seeds producing oil comprised of 70-80% of the respective fatty acid (GRDC, 2017; Singh and Nimbkar, 2006).

25. Safflower is naturalised in Australia. Records of safflower populations naturalising in several global ecosystems indicates safflower has high risk weed potential (Randall, 2017). However, safflower is considered a minor problem in natural ecosystems, and is not considered to warrant control in agricultural ecosystems (Groves et al., 2003). The Victorian weed list gives safflower a risk ranking score of zero and classifies it as 'lower risk' (White et al., 2022). Safflower lacks several inherent agronomic qualities which contribute to a tendency to weediness, including seed dormancy, high seed output, high seed dispersal, long-distance seed dispersal, seed shattering, persistent seed banks, and rapid growth to flowering (OECD, 2022). Therefore, given its poorly competitive nature and slow growth rate, safflower has limited capacity to invade in undisturbed natural areas (OGTR, 2019).

26. Safflower is primarily self-pollinated (85-90%), with the remaining pollination (10-15%) by insects, primarily honey bees (*Apis mellifera* L.) (Knowles, 1969; Rubis, 1970). Pollination rarely occurs via wind, as safflower pollen is not carried by wind beyond 1 m (Claassen, 1950). Safflower outcrossing occurs at a rate of approximately 10% (GRDC, 2017), although outcrossing between safflower plants in close proximity (1-1.5m) is highly variable (Claassen, 1950; Knowles, 1969). One study investigating outcrossing of GM to non-GM safflower in Canada observed outcrossing frequencies of 1.7% between safflower plots 3 m apart, 0.01% at 100 m, and no outcrossing detected at 300 m (McPherson et al., 2009a). Cross-pollination and seed set can be increased by insect pollinators (Claassen, 1950; GRDC, 2017), with wind-mediated outcrossing playing a minor role. Other abiotic and biotic factors relating to safflower are discussed in Section 5.

27. Safflower seeds are firmly contained in the safflower head, which are highly resistant to shattering, limiting access to seed by small animals such as rodents (OGTR, 2019). Seeds are smooth and relatively large (approximately 7 mm long and 40 mg in weight), with each safflower head containing 15-60 seeds (OECD, 2022). Safflower seeds have very low dormancy, and mature seeds may germinate in the flower head following excess rainfall or high humidity (GRDC, 2017; Zimmerman, 1972), so seeds that fall to the ground during harvest are expected to germinate readily. However, another study reported that persistence of viable safflower seed at the soil surface was approximately 2 years, while viable seed persisted for approximately 6 months when buried in the soil (McPherson et al., 2009b). Furthermore, long-term storage (24 weeks) reduced safflower seed dormancy (Kotecha and Zimmerman, 1978).

28. Large animals such as pigs and kangaroos are deterred from grazing safflower by the hard spines present on the upper leaves of safflower plants (GRDC, 2017). Safflower seed is consumed by bird species. Safflower seed is destroyed and non-viable after passing through the digestive tracts of mallards, pheasants, blackbirds and pigeons (Cummings et al., 2008). However, seed may be stored in the oesophagus or gizzard of these birds for several hours, and viable seed may be regurgitated, although with reduced ability to germinate (Cummings et al., 2008). Safflower seeds do not possess adaptations such as hooks or spines, which limits the potential for dispersal via attachment to the exterior of animals (Mayerhofer et al., 2011), except if attached to an animal via heavy soil.

29. Safflower seed oil and meal are not considered to be toxic and have a long history of safe use. Safflower seeds and meal contain some anti-nutritional factors and toxins, such as cyanogenic glycosides, tannins and oxalates (Singhal et al., 2019), however their levels are considered non-toxic to rats (Ingale and Shrivastava, 2011). The high fibre content of the seeds renders seed and seed meal unpalatable and difficult to digest by animals, limiting its use in livestock feed. Safflower petal extracts have been used in Chinese herbal medicine for centuries and there are many reports of the beneficial effects of safflower in the treatment of several medical conditions (Cheng et al., 2024; Zhou et al., 2014). Some reports suggest extract from safflower petals is cytotoxic (Mohseni et al., 2011) or nephrotoxic (Liu et al., 2004) in animal studies, though more recent studies indicate no adverse effects on fertility or development in rats (Lewin et al., 2021).

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

30. The applicant proposes to release 12 groups of up to 10 safflower lines each, genetically modified for dairy protein production and altered fat composition.

4.1 The genetic modifications in the GMOs proposed for release

31. Two safflower breeding lines (X3463-007 and/or X3463-009) were used as the recipients for transformation to generate the GM safflower lines proposed for release in this application.

32. All GM safflower lines will contain one of 2 variants (denoted BCN2 and BCN3) of the gene encoding β-casein (*CSN2*) from domestic cattle, fused to a gene derived from common plant species. Some lines contain sequences to target the expressed β-casein fusion proteins to the cell wall or vacuoles. Some lines contain an inverted repeat to drive down-regulation of fatty acid desaturase genes *SAD* or *FAD2.2* via RNA interference (RNAi). All GM safflower lines contain either an antibiotic (hygromycin) or glufosinate herbicide tolerance marker gene. One group of GM safflower lines also contains the β-glucuronidase (GUS) visual selection reporter gene. Genes introduced into the GM safflower lines are summarised in Table 3. Regulatory and localisation sequences are summarised in Table 4. Each GM safflower line contains only a subset of the genetic elements listed in Table 3 and Table 4.

33. The identities of some the genetic elements, and the arrangement of all genetic elements in the vectors, introduced into GM safflower have been declared Confidential Commercial Information (CCI). Under section 185 of the Act, the confidential information is made available to the prescribed agencies and experts that are consulted on the RARMP for this application.

Table 3. Introduced genes

Genetic element	Source organism	Encoded protein	Intended function
bar	*Streptomyces hygroscopicus*	Phosphinothricin acetyltransferase	Herbicide resistance
BCN2	*Bos taurus*	Miruku β-casein (CSN2) variant 2	β-casein production
BCN3	*Bos taurus*	Miruku β-casein (CSN2) variant 3	β-casein production
CCI gene	CCI (common plant species)	CCI	CCI
CCI gene	CCI (common plant species)	CCI	CCI
CtFAD2.2 fragments	*Carthamus tinctorius*	Hairpin RNA targeting *FAD2.2* desaturase for RNAi-mediated gene silencing	*FAD2.2* down-regulation
Cat-1 intron	*Ricinus communis*		
CtSAD fragments	*C. tinctorius*	Hairpin RNA targeting *SAD* desaturase for RNAi-mediated gene silencing	*SAD* down-regulation
Cat-1 intron	*R. communis*		
GUSPlus^{TM}	*Staphylococcus* sp.	Codon-optimised β-glucuronidase derived from *gusA* gene	Visual selectable marker
hph	*S. hygroscopicus*	Hygromycin B phosphotransferase	Antibiotic resistance

4.1.1 BCN2 and BCN3

34. The introduced *BCN2* and *BCN3* constructs encode variants to A2 β-casein from cattle (*Bos taurus*). The purpose of the introduction of *BCN2* and *BCN3* is to produce the β-casein protein variants in seeds of GM safflower. Expression is driven by one of 2 seed-specific promoters, either alone, or in combination with organelle localisation sequences.

35. Caseins are the primary proteins in bovine milk, comprising 80% of total protein content (Hassanin et al., 2022). β-casein accounts for approximately 35% of bovine milk protein (Daniloski et al., 2022), and is highly polymorphic, with at least 15 known variants (Sebastiani et al., 2020). The A1 and A2 variants are the most common variants, which differ by a single amino acid at residue 67 (Farrell et al., 2004). β-casein has a highly charged and hydrophilic N-terminal region and a primarily hydrophobic C-terminus (Dauphas et al., 2005), which is primarily due to the presence of 5 phosphorylated serine residues (Creamer et al., 1981; McCarthy et al., 2013). This amphiphilic nature enables β-casein to act as an emulsifier, and partial dephosphorylation of these serine residues results in reduced ability of β-casein to stabilise emulsions and the accumulation of β-casein in larger globules (Cassiano and Areas, 2003; McCarthy et al., 2013).

36. The *BCN2* and *BCN3* coding sequences are identical to the A2 β-casein sequence, except *BCN2* replaces serine residues at positions 17, 19 and 35 with aspartic acid, while *BCN3* replaces serine residues at positions 15, 18 and 35 with aspartic acid and serine residues at positions 17 and 19 with glutamic acid. These modifications are expected to result in partial dephosphorylation of β-casein and alter its emulsification properties.

37. *BCN2* and *BCN3* coding sequences are fused with a gene derived from common plant species and are expressed as a chimeric fusion protein using a seed-specific promoter.

4.1.2 CtSAD/CtFAD2.2 fragments and Cat-1 intron

38. *SAD* and *FAD2* are fatty acid desaturase genes which convert saturated fatty acids to unsaturated fatty acids (Rajwade et al., 2014). SAD desaturates stearic acid to form oleic acid, and FAD2 further desaturates oleic acid to form linoleic acid (Dong et al., 2024). In safflower seeds, fatty acid composition is 71-75% linoleic acid, 16-20% oleic acid, 6-8% palmitic acid, and 2-3% stearic acid (Deliorman Orhan et al., 2022).

39. The applicant is proposing to down-regulate *SAD* and *FAD2* expression in safflower seeds using fragments of these genes (*CtSAD* and *CtFAD2.2* fragments) for RNAi. RNAi utilises an inserted gene construct consisting of inverted repeat fragments of a gene sequence which is processed by the endogenous cellular machinery to form short interfering RNAs (siRNAs). The siRNAs degrade complementary *SAD* or *FAD2* messenger RNA (mRNA) transcripts to result in sequence-specific gene silencing (Koeppe et al., 2023). Down-regulating expression of *SAD* is expected to decrease the proportion of oleic acid and increase levels of stearic acid, while down-regulating expression of *FAD2* is expected to decrease the proportion linoleic acid and increase levels of oleic acid. *CtSAD* and *CtFAD2.2* fragments will not be inserted into GM safflower lines together.

40. Efficiency of gene silencing is generally determined by the degree of homology between the RNAi fragments and the target gene (Yan et al., 2020). 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 (Yan et al., 2020).

41. RNAi efficiency can be improved via inclusion of a non-complementary space sequence between the complementary RNAi fragments (Asadi et al., 2024). The applicant proposes to include the *Cat-1* intron sequence from *Ricinus communis CtSAD* or *CtFAD2.2* fragments. Intron DNA sequence itself is not expressed as a protein, and inserted introns are expected to operate in similar ways to endogenous sequences.

42. RNAi-mediated gene silencing of *SAD* and *FAD2* is achieved by expressing the inserted *CtSAD* and *CtFAD2.2* fragments in safflower seeds using seed-specific promoters in order to alter the fatty acid composition of GM safflower seeds. A previous study utilised RNAi to down-regulate *FAD2* and a thioesterase gene in safflower seeds to produce seeds with altered fatty acid composition consisting of 93% oleic acid (Wood et al., 2018). These studies were the subject of limited and controlled releases of GM safflower approved by the Regulator (DIR 121 and DIR 131).

4.1.3 bar

43. The *bar* (*bialaphos resistance*) gene is derived from the bacterium *Streptomyces hygroscopicus* (Thompson et al., 1987). The *bar* gene encodes a phosphinothricin acetyltransferase (PAT) enzyme that

confers tolerance to glufosinate herbicide. PAT acetylates glufosinate, converting it to *N*-acetyl-L-glufosinate, which is not toxic to plants (OECD, 2002).

44. Expression of *bar* is controlled by a 35S promoter from the Cauliflower mosaic virus (*CaMV35S*), which drives constitutive expression of *bar* in all plant tissues (Kay et al., 1987).

45. The Regulator has previously assessed and approved GM crops containing the *bar* gene for commercial release in Australia, most recently under licence DIR-190, and for field trials, most recently under DIR 204.

4.1.4 hph

46. The *hph* gene inserted into GM safflower is derived from the bacterium *S. hygroscopicus* and encodes the enzyme hygromycin B phosphotransferase, which confers resistance to the antibiotic hygromycin (Pardo et al., 1985). Expression of *hph* is controlled by the constitutive promoter *CaMV35S*.

47. The *hph* gene was used as a marker in the laboratory to select for GM safflower transformants during early stages of development.

48. The Regulator has previously assessed and approved GM crops containing the *hph* gene for commercial release in Australia, most recently under licence DIR 158, and for field trials most recently under DIR 160. More information on marker genes, including *hph*, may be found in the document Marker Genes in GM Plants which is available from the OGTR website.

4.1.5 GUSPlus™

49. *GUSPlus* is a codon-optimised *gusA* gene based on the sequence from *Staphylococcus* sp., which encodes an enhanced β-glucuronidase detectable at levels as much as 10-fold lower than *E. coli* GUS (Broothaerts et al., 2005; Vickers et al., 2007). *GUSPlus* is a visual maker used to identify successful transformants (Jefferson et al., 1986).

50. In the GM safflower, expression of *GUSPlus* is controlled by the constitutive promoter *CaMV35S*. *GUSPlus* was used as a marker to monitor efficiency of GM safflower transformation in the laboratory during early stages of development.

4.1.6 Regulatory and localisation sequences

51. The GM safflower lines will also contain introduced regulatory sequences and localisation signals to control expression of the inserted genes (Table 4). These include promoters to drive gene expression, terminators, and localisation signals to spatially restrict protein localisation. The identities of some of the promoters and 3'UTR sequences introduced into GM safflower have been declared CCI by the Regulator. Under section 185 of the Act, the CCI has been made available to the prescribed agencies and experts that are consulted on the RARMP for this application.

Table 4. Introduced regulatory elements and localisation sequences

Genetic element	Source	Intended function
AtCel1 cell wall signal	Cell wall signal from *Arabidopsis thaliana CEL1* gene	Cell wall targeting sequence
CaMV35S 3'UTR	Cauliflower mosaic virus	Terminator and polyadenylation signal
CaMV35S promoter	Cauliflower mosaic virus	Constitutive promoter
CCI 3'UTR 1	*Glycine max*	Terminator and polyadenylation signal
CCI 3'UTR 2	*Phaseolus vulgaris*	Terminator and polyadenylation signal
CCI 3'UTR 3	*Linum usitatissimum*	Terminator and polyadenylation signal

Genetic element	Source	Intended function
CCI promoter 1	*G. max*	Seed-specific promoter
CCI promoter 2	*P. vulgaris*	Seed-specific promoter
CCI promoter 3	*L. usitatissimum*	Seed-specific promoter
CCI promoter 4	*Brassica napus*	Seed-specific promoter
CT-CVS C-terminus	C-terminal beta-conglycinin vacuolar sequence from *Glycine max*	Terminator
ER Retention	C-terminal H/KDEL tag sequence from *A. thaliana*	Endoplasmic reticulum retention
GmCVS vacuolar signal	Vacuole signal from *G. max* beta-conglycinin gene	Vacuole targeting sequence
Goshu-lectin 3'UTR	*Dioscorea batatas*	Terminator and polyadenylation signal
HvAVSP vacuolar signal	Vacuole signal from *Hordeum vulgare* aleurain gene	Vacuole targeting sequence
Prr	*Nicotiana tabacum*	Cell wall targeting sequence
nos 3'UTR	*Agrobacterium tumefaciens*	Terminator and polyadenylation signal
nos promoter	*A. tumefaciens*	Constitutive promoter

4.2 Method of genetic modification

52. The GM safflower lines were generated by *Agrobacterium*–mediated transformation. This method has been widely used in Australia and overseas for introducing genes into plants. Information about this method can be found in the document Methods of plant genetic modification, available from the OGTR Risk Assessment References page.

53. Safflower breeding lines X3463-007 and/or X3463-009 were transformed using the methodology described by Belide et al. (2011). Transformants were selected on media containing either the antibiotic hygromycin B or the herbicide glufosinate. Selection agents were also used to eliminate *Agrobacterium* during *in vitro* selection of the transformed safflower plants. *Agrobacterium* is not normally transmitted from one generation to the next via seed, therefore selected GM safflower plants were propagated by single seed descent.

54. Parental safflower lines were transformed with one of 12 binary plasmid vectors classified into 9 categories, which are described in Table 5. The applicant initially proposed release of 10 categories of safflower lines, however has since withdrawn application for release of category 7. The arrangement of the genetic elements in the remaining 12 vectors in 9 categories introduced into GM safflower have been declared CCI.

Table 5. Categories of binary vector transformed into GM safflower

Category	Vector number	Description
1	1	β-casein version 2 fusion (BCN2) fusion protein with glufosinate resistance
2	2-5	β-casein version 3 fusion (BCN3) fusion protein with various cell wall or vacuole targeting sequences and glufosinate resistance
3	6	BCN3 fusion protein with glufosinate resistance
4	7	BCN3 fusion protein with down-regulation of fatty acid desaturase and glufosinate resistance

Category	Vector number	Description
5	8	BCN3 fusion protein with beta-glucuronidase (GUS) and hygromycin resistance
6	9	BCN3 fusion protein with down-regulation of stearoyl-acyl carrier protein desaturase and glufosinate resistance
8	11	BCN3 fusion protein with hygromycin resistance
9	12	BCN3 fusion protein with down-regulation of fatty acid desaturase and hygromycin resistance
10	13	BCN3 fusion protein with down-regulation of stearoyl-acyl carrier protein desaturase and hygromycin resistance

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

55. As the GMOs are at an early stage of development, no toxicity or allergenicity studies have been conducted on the GM safflower plants or purified protein produced by the introduced genetic elements. The genetic element components of the β-casein fusion protein and their encoded proteins have also not been assessed by authorities in any country for toxicity and allergenicity.

56. Discussion of the toxicity/allergenicity of some the introduced genes that have been declared CCI is made available to the prescribed agencies and experts that are consulted on the RARMP for this application as required under section 185 of the Act.

4.3.1 β-casein

57. A recent population-based study reported cow's milk allergy (CMA) in 1.3% of 1-year-old Australian infants (Soriano et al., 2023). Allergic reactions to cow's milk proteins are characterised by asthma, atopic dermatitis, urticaria (hives), rhinitis, gastrointestinal disorders, and anaphylaxis (Docena et al., 1996). Tolerance to cow's milk proteins usually develops as children mature, and consequently is present in less than 0.5% of adults (Fiocchi et al., 2010).

58. Caseins are known food allergens. In one study of 80 patients with known CMA, 100% of patients produced immunoglobulin-E (IgE) antibodies against casein proteins, indicating an immune reaction (Docena et al., 1996), while in another study of CMA patients, 75.3% produced IgE antibodies against caseins (Shoormasti et al., 2011).

59. Little information was found regarding potential toxicity or allergenicity of β-casein specifically. Upon digestion, proteolysis of the A1 variant of β-casein produces the peptide β-casomorphin-7 (BCM-7), which is implicated in adverse gastrointestinal effects (Giribaldi et al., 2022). However, the applicant proposes to introduce sequence variations to the A2 variant in GM safflower, which does not produce BCM-7 and consequently is not associated with digestive intolerance. Nonetheless, given the high rate of allergenicity to caseins among CMA patients, (Daniloski et al., 2022), allergenicity to A2 β-casein variants is possible.

60. Allergenicity to caseins via dermal contact is rare, and often requires existing damage to the skin to cause an immune reaction (Jensen et al., 2022). One study describes a single patient who developed dermatitis and rhinitis in response to casein protein present in microbiology laboratory culture media (Nakonechna et al., 2019). In another study, a single patient developed rhinitis and asthma following exposure to casein in a dermatological formulation associated with their occupation (Bonadonna et al., 2003). No information was found in the literature on toxicity or allergenicity associated with dermal contact with β-casein specifically.

4.3.2 RNAi gene silencing constructs for SAD and FAD2

61. Insertion of fragments of safflower genes *SAD* and *FAD2* as part of gene silencing constructs does not result in expression of a protein, but instead suppresses expression of endogenous safflower proteins. Similarly, the *Cat-1* intron is inserted as a spacer sequence to facilitate formation of the RNAi hairpins and

does not encode an expressed protein. Therefore, these genetic elements are not expected to lead to increased toxicity or allergenicity.

62. The effect of the gene silencing is to increase levels of either stearic acid or oleic acid relative to other fatty acids in GM safflower seeds. Stearic acid is a commonly consumed by humans as part of a regular diet. It is a component of meat, eggs, dairy, grains, legumes and oils, and is the second most consumed saturated fatty acid via the diet in the United States (Hunter et al., 2010). Contact allergy to stearic acid is very rare. There is a single report in the literature of an allergic reaction to stearic acid in a cosmetic product (de Groot et al., 1988). Otherwise, stearic acid is not associated with allergenicity or toxicity, except when consumed in excess, where it is correlated with an increased risk of cardiovascular disease (Hunter et al., 2010).

63. Oleic acid is the main fatty acid found in animal fats and in vegetable oils such as olive oils, and accounts for approximately 90% of monounsaturated fatty acids in the average human diet (Schwingshackl and Hoffmann, 2014). Oleic acid is associated with health benefits and it is not associated with toxicity or allergenicity (Sales-Campos et al., 2013).

64. Despite containing some anti-nutritional factors, 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; Toma et al., 2014).

4.3.3 PAT protein

65. The *bar* gene and its encoded PAT protein have been extensively assessed in previous RARMPs for commercial release of GM crops including canola (DIR 021/2002, DIR 108, DIR 138, DIR 175), cotton (DIR 062/2005, DIR 143, DIR 145, DIR 173) and a limited and controlled release of GM wheat (DIR 204). The PAT protein has been assessed to lack toxicity to humans or animals, or allergenicity in humans on the following basis:

- the *bar* gene was derived from the common soil bacterium *S. hygroscopicus*, which is not considered a pathogen of humans or other animals;
- no sequence homology has been found between PAT and any known toxic or allergenic proteins;
- the PAT protein does not possess any of the characteristics associated with food allergens;
- the PAT protein is inactivated by heat, e.g. through cooking, and by low pH, e.g. in the human stomach;
- the PAT protein is rapidly degraded in simulated gastric or intestinal fluid; and
- purified PAT protein was not toxic to mice and rats when administered at high doses in acute toxicity studies.

66. FSANZ has approved food derived from a number of GM crops expressing PAT protein as safe for human consumption. This includes GM canola (ANZFA, 2001; FSANZ, 2017), cotton (FSANZ, 2005a, 2010a, b, 2013a), corn (FSANZ, 2005b) and rice (FSANZ, 2008).

4.3.4 hph

67. The risks of hph 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 hph is toxic or allergenic to humans.

4.3.5 GUSPlus™

68. The toxicity or allergenicity of *GUSPlus™* to humans or animals has not been assessed previously. However, the potential risks of the endogenous bacterial GUS protein, from which the sequence of *GUSPlus™* is derived, 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 GUS is toxic or allergenic to humans.

4.4 Characterisation of the GMOs

69. The GM safflower lines proposed for release are still in development. Initial observations of the GM safflower grown in controlled glasshouse conditions indicated no phenotypic differences compared to non-GM safflower. Both GM and non-GM safflower exhibited similar growth patterns, morphology, germination rates, time to flowering, plant height, seed count per plant and seed oil content. No quantitative data were provided by the applicant. Further data on the agronomic performance of the GM safflower lines is proposed to be collected during the field trials.

Section 5 The receiving environment

70. 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 GMOs; and background presence of the gene(s) used in the genetic modification (OGTR, 2013).

71. Detailed information regarding factors relevant to the growth, distribution and cultivation of safflower in Australia can be found in *GRDC Safflower GrowNotes* (GRDC, 2017) and *The Biology of* Carthamus tinctorius *L. (safflower)* (OGTR, 2019) and is summarised below.

5.1 Relevant abiotic factors

72. In Australia, safflower is an annual plant with a long growing season. It is best adapted to the cereal growing regions of southern NSW, Vic and SA with higher rainfall (>450 mm), a dry climate during late spring and early summer, and stored subsoil water reserves. It is relatively drought tolerant. Safflower does not tolerate waterlogging, as this can starve roots of oxygen and encourage the development of fungal diseases (GRDC, 2017).

73. 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^{o}C. Mean daily temperatures above 26^{o}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, 2019). Other abiotic stresses that can reduce yield and oil content include susceptibility of young plants to hail damage (OGTR, 2019).

5.2 Relevant biotic factors

74. In Australia, there are a number of common insect pests, as well as some minor pest species. Main insect pests can all be controlled with insecticides and some with biological controls (GRDC, 2017). Safflower is most susceptible to damage by insects during establishment and between budding and harvest (GRDC, 2017).

75. 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 that harbour birds (GRDC, 2017).

76. 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). Safflower in Australia is affected by a range of fungal diseases (GRDC, 2017). Control of disease in Australia relies on agricultural practices (OGTR, 2019).

77. Safflower is a poor competitor with weeds, due to slow growth at the rosette stage early in the season (GRDC, 2017). Later in the season many weeds can shade safflower plants and significantly reduce crop yields (GRDC, 2017; Li and Mündel, 1996). However, knockdown herbicides or cultivation prior to sowing can be used for weed control (GRDC, 2017). Safflower is tolerant of some herbicides, but the number of herbicides available for use in Australia is limited (GRDC, 2017). See the APVMA PubCris database for more information on registered herbicides for weed control in safflower.

5.3 Relevant agricultural practices

78. The applicant specifies that GM safflower seeds would be planted in trial sites during the winter cropping season, but a summer cropping cycle may also be used occasionally. Non-GM safflower lines planted at the trial sites for comparative purposes would be treated as if they were GM safflower.

79. GM and non-GM safflower crops would be maintained in a similar manner to commercial safflower crops, except the applicant proposes to restrict the dispersal and persistence of the GM safflower (see Section 2.2). Standard cultivation practices for safflower in Australia are discussed in *GRDC Safflower GrowNotes* (GRDC, 2017).

80. The applicant proposes to only use the glufosinate tolerance conferred by the introduced *bar* gene as a selectable marker during transformation. Glufosinate herbicide is not intended to be applied to plants growing in the field trial.

81. The applicant specifies that the GM safflower would be grown at field sites either as an irrigated or dryland crop. Seeds would be planted in row plots with typical row spacing for safflower, e.g. 30-40 cm, in plots spaced 1-2 m apart although other configurations may be used. Small areas would be hand-planted or planted with a small plot cone-seeder, while larger areas would be planted with commercial equipment.

82. Nitrogen fertiliser would be deep injected pre-plant or at planting. Land would be cultivated once or twice after planting to control weeds, aerate soil and allow efficient irrigation. Furrow or flood irrigation would be used where necessary, and pre-irrigation may be conducted to store soil moisture and reduce salt levels in the soil.

83. Pest monitoring would be conducted once or twice per week by field technicians.

84. Safflower seeds would be harvested by hand for small plantings or with commercial equipment for larger plantings, when seed moisture reaches 5-8%. Safflower typically takes 110-150 days from planting to harvest.

85. Planting areas will be left fallow after harvest and site cleaning to facilitate the germination and monitoring of volunteers. Sites may be replanted with GM safflower in subsequent years or would be planted with rotation crops such as cereals or pulses.

86. Additional agricultural practices proposed by the applicant are discussed further in Chapter 3, Section 3.1.

5.4 Presence of related plants in the receiving environment

87. Safflower is a minor commercial crop in Australia, primarily grown in SA, NSW and Vic (ABARES, 2024). Naturalised populations of wild safflower have been reported at low levels in all states and territories of Australia (Atlas of Living Australia, accessed 29 January 2025). Wild safflower is considered a minor weed that primarily establishes on disturbed ground (Groves et al., 2003). Currently 2 GM safflower lines are approved by the Regulator for commercial cultivation in Australia, under licence DIR 158.

88. There are 4 related *Carthamus* species reported as present in Australia: *C. lanatus, C. leucocaulos*, *C. dentatus* and *C. glaucus*, although there is some doubt about the existence of *C. glaucus* in Australia. All 4 species have a chromosome number of n=10, whereas for safflower (*C. tinctorius*) n=12. These related species have all been reported as naturalised in Australia (Atlas of Living Australia, accessed 29 January 2025). Both *C. lanatus* and *C. leucocaulos* have been declared agricultural weeds in some states or territories (Weeds Australia, accessed 29 January 2025). Under controlled conditions, *C. leucocaulos* and *C. lanatus* can cross with *C. tinctorius* but produce sterile F_1 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). Studies demonstrate that sterile safflower plants can lack pollen (Heaton and Knowles, 1982; Kammili and Morris, 2013), suggesting that sterile offspring from hybrid crosses between safflower and related species could also lack pollen.

5.5 Presence of similar genes and their products in the environment

89. *BCN2* and *BCN3* are modified sequences of the gene *CSN2* derived from cattle (*Bos taurus*), which is naturally present in the environment. The genetic sequence of the other part of the fusion protein constituent is derived from common plant species and these sequences naturally occur in all plants. The *CtSAD* and *CtFAD2.2* gene fragments are derived from endogenous safflower genes naturally present in all safflower plants.

90. The *hph* and *bar* genes are from the common soil bacterium *S. hygroscopicus*, which is widespread and prevalent in the environment. *GUSPlus*^{TM} is derived from the *gusA* gene from *Staphylococcus* sp., which is prevalent in the environment, including in the human digestive system.

91. Regulatory and localisation sequences are derived from common plants, a plant virus (CaMV) or a soil bacterium (*A. tumefaciens*) that are widespread in the environment. Although some of the regulatory sequences are derived from plant pathogens (*A. tumefaciens* and CaMV), they comprise only small parts of the total genomes and cannot of themselves cause disease.

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

Approvals by the Regulator

92. The GM safflower lines included in this application have not been previously approved for release in Australia.

Approvals by other government agencies

93. The GM safflower lines included in this application have not been previously approved by any other government agency in Australia.

6.2 International approvals

94. The GM safflower lines included in this application have not received any approvals from authorities in other countries.

Chapter 2 Risk assessment

Section 1 Introduction

95. 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 3). Risks are identified within the established risk assessment context (Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

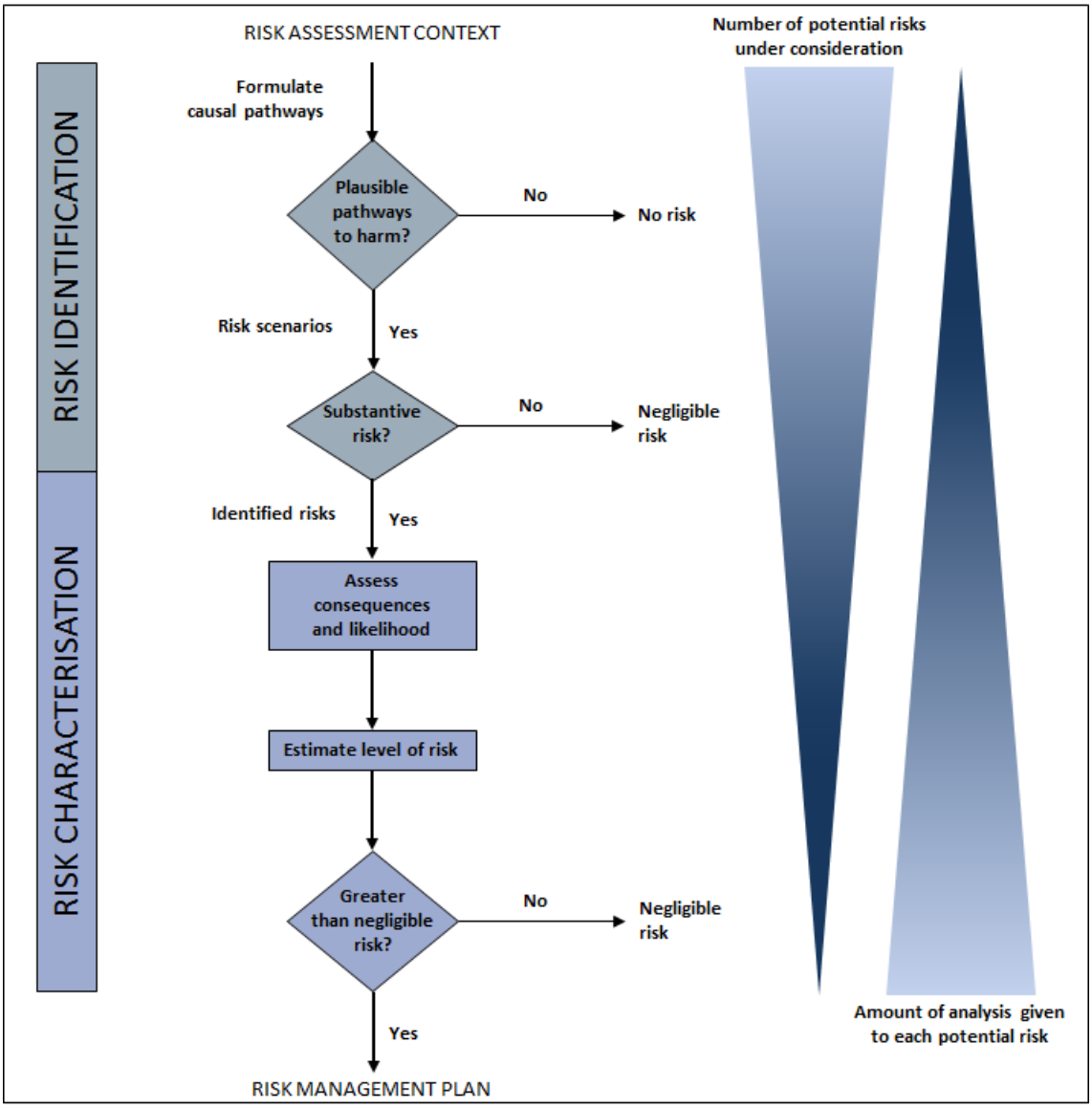

Figure 3. The risk assessment process

96. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, reported international experience and consultation (OGTR, 2013).

97. Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating plausible causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are called risk scenarios.

98. Risk scenarios are screened to identify substantive risks, which are risk scenarios that are considered to have some reasonable chance of causing harm. Risk scenarios that could not plausibly occur, or do not lead to harm in the short and long term, do not advance in the risk assessment process (Figure 3), i.e. the risk is considered to be no greater than negligible.

99. Risk scenarios identified as substantive risks are further characterised in terms of the potential seriousness of harm (consequence assessment) and the likelihood of harm (likelihood assessment). The consequence and likelihood assessments are combined to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

100. A weed risk assessment approach is used to identify traits that may contribute to risks from GM plants, as this approach addresses the full range of potential adverse outcomes associated with plants. In particular, novel traits that may increase the potential of the GMO to spread and persist in the environment or increase the level of potential harm compared with the parental plant(s) are used to postulate risk scenarios (Keese et al., 2014). Risk scenarios postulated in previous RARMPs prepared for licence applications for the same or similar GMOs, are also considered.

Section 2 Risk identification

101. Postulated risk scenarios are comprised of 3 components (Figure 4):

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.

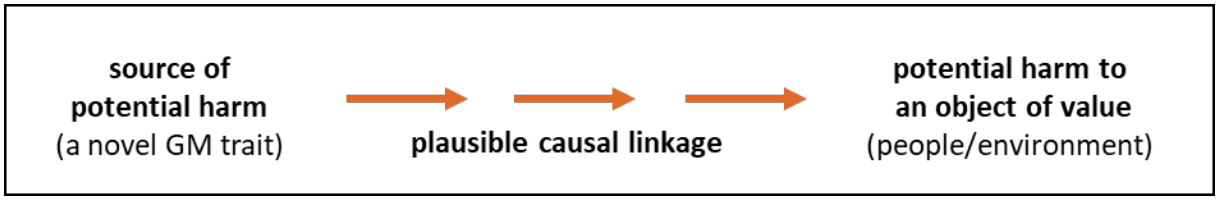

Figure 4. Components of a risk scenario

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

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

2.1 Risk source

103. 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.

104. As discussed in Chapter 1, the GM safflower lines have been modified by the introduction of fusion protein sequences comprised of variants of *CSN2* from cattle and a gene derived from common plant species intended to produce dairy protein in seed. Some GM safflower lines have been modified by the introduction of gene silencing constructs derived from safflower and intended to alter fatty acid profiles of seeds. These introduced genes are considered further as sources of potential harm.

105. The GM safflower lines also contain the *hph* or *bar* genes and in one line, codon-optimised *gusA* gene. These genes confer hygromycin antibiotic resistance, glufosinate herbicide tolerance and visual marker expression, respectively, and were used as selectable marker genes. These genes and their products have been extensively characterised and assessed as posing negligible risk to human or animal health or to

the environment by the Regulator, as well as by other regulatory agencies in Australia and overseas. As the genes have not been found to pose a substantive risk to either people or the environment, their potential effects will not be further considered for this application.

106. The introduced genes are controlled by introduced regulatory sequences derived from various species (see Table 4). The GM safflower may also contain intron DNA sequences. Regulatory sequences and introns are naturally present in all plants, and the introduced sequences are expected to operate in similar ways to endogenous sequences. The regulatory sequences and introns are DNA that is not expressed as a protein, so exposure is to the DNA only and dietary DNA has no toxicity (Delaney et al., 2018). Hence, potential harms from the regulatory sequences and introns will not be considered further. However, seed specificity of promoters will be discussed in the context of other risk sources.

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

2.2 Causal pathway

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

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

109. Although all of these factors are taken into account, some are not included in risk scenarios because they have been considered in previous RARMPs and are not expected to give rise to substantive risks.

110. The potential for horizontal gene transfer (HGT) from GMOs to species that are not sexually compatible, and any possible adverse outcomes, have been reviewed in the literature (Keese, 2008; Philips et al., 2022) and assessed in previous RARMPs. No risk greater than negligible was identified, due to the rarity of HGT events and because the gene sequences are already present in the environment and available for transfer via demonstrated natural mechanisms. Therefore, HGT will not be assessed further.

111. 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 the 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

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

- 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).

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

2.4 Postulated risk scenarios

114. Four risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 6 and examined in detail in Sections 2.4.1 – 2.4.4.

115. In the context of the activities proposed by the applicant and considering both the short and long term, none of the 4 risk scenarios gave rise to any substantive risks.

Table 6. Summary of risk scenarios from the proposed dealings with GM safflower

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced genes for dairy protein production and altered fat composition	Cultivation of GM safflower at trial sites ⬇ Exposure of people and desirable animals to products of the introduced genes	Increased toxicity or allergenicity for people OR increased toxicity to desirable animals	No	• The GM safflower would not be used as commercial human food or animal feed • The short duration and proposed controls for the field trial would restrict exposure to and consumption of GM plant material by animals • The limits and controls of the field trial would restrict exposure of people to the GM plants • β-casein fusion proteins are not expected to be toxic but could be allergenic. However, people will not consume GM safflower seeds or oil (other than a small number of people as part of the sensory tests), and animals are unlikely to consume a dose required for toxicity • Insertion of gene silencing constructs does not lead to expression of a protein.
2	Introduced genes for dairy protein production and altered fat composition	Cultivation of GM safflower at trial sites ⬇ Persistence of GM safflower seed at trial sites or dispersal of GM seed outside trial limits ⬇ Establishment of populations of volunteer GM plants expressing the introduced genes in the environment	Increased toxicity or allergenicity for people OR increased toxicity to desirable animals OR reduced establishment or yield of desirable plants	No	• The limits and controls of the field trial would minimise dispersal or persistence of GM seeds • GM safflower is susceptible to standard weed management measures • As discussed in Risk Scenario 1, no substantive risk was identified for increased toxicity or allergenicity of the GM safflower • Safflower has limited ability to compete with other plants and the genetic modifications are not expected to increase the overall weediness or competitiveness of the GM safflower.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
3	Introduced genes for dairy protein production and altered fat composition	Cultivation of GM safflower at trial sites ⬇ Pollen from GM plants dispersed outside the trial sites ⬇ Outcrossing with sexually compatible plants ⬇ Establishment of populations of hybrid GM plants expressing the introduced genes in the environment	Increased toxicity or allergenicity for people OR increased toxicity to desirable animals OR reduced establishment or yield of desirable plants	No	• The controls of the field trial would minimise pollen flow to sexually compatible plants outside the trial sites • Safflower pollen has limited ability to disperse over long distances • In Australia, safflower is either not sexually compatible with related species, or produces sterile hybrids • As discussed in Risk Scenario 1, no substantive risk was identified for increased toxicity or allergenicity of the GM safflower • As discussed in Risk Scenario 2, the genetic modifications are not expected to increase the overall competitiveness of the GM safflower with other plants.
4	Introduced genes for dairy protein production and altered fat composition	Cultivation of GM safflower at trial sites ⬇ Consumption of GM safflower seed by pest animals ⬇ Increased fitness of pest animals	Reduced establishment of yield of desirable plants OR Reduced biodiversity	No	• The limited scale and other proposed limits and controls minimise exposure of pests to the GM seeds • GM safflower seeds are unlikely to contribute a large proportion of the overall diet for pest species • Pests are controlled by current pest management practices.

2.4.1 Risk scenario 1

Risk source	Introduced genes for dairy protein production and altered fat composition
Causal pathway	⬇ Cultivation of GM safflower at trial sites ⬇ Exposure of people and desirable animals to products of the introduced genes ⬇
Potential harm	Increased toxicity or allergenicity for people OR Increased toxicity to desirable animals

Risk source

116. The source of potential harm for this postulated risk scenario is the introduced genes for dairy protein production and altered fat composition in GM safflower plants.

Causal pathway

117. The GM safflower would be grown at the trial sites. As the introduced genes for dairy protein production and altered fatty acid composition are controlled by seed-specific promoters (see Table 4), the encoded fusion proteins and gene silencing constructs would be produced in the seeds of the GM plants. The names of these promoters have been declared CCI by the Regulator. Information about the promoters has been made available to the prescribed agencies and experts that are consulted on the RARMP under section 185 of the Act.

118. β-casein fusion proteins will be expressed under the control of one of 2 promoters. A review of the literature on these promoters corroborates seed-specific expression for one of these promoters. A study utilising this promoter to express an introduced gene in a common plant species showed expression in seeds but not in leaf tissue. However, the other promoter used to drive the β-casein fusion proteins may have leaky expression. One study showed that this promoter is not active in vegetative tissues such as leaves, stems and roots, but is active in both seeds and anthers of a transgenic plant. As pollen is produced in the anthers, this promoter may generate pollen containing β-casein fusion proteins. Therefore, people working on the trial site or in the vicinity of the trial site could inhale airborne pollen during flowering of the GM safflower. However, safflower pollen is not carried by wind beyond 1 m (Claassen, 1950), thus exposure to pollen containing fusion proteins would be limited. The applicant has not tested the seeds or any other tissues for levels of produced fusion proteins.

119. The gene silencing constructs *CtSAD* and *CtFAD2.2* will be expressed under the control of one of 2 seed-specific promoters. An analysis of regulatory elements within the promoter sequence of one of these promoters demonstrates strong spatial restriction to seeds, while patent data showed a very small amount of expression in buds. Similarly, a study supports the seed-specific activity in transgenic plants for the other promoter used to drive the *CtSAD* and *CtFAD2.2* constructs. Trial staff working at sites with GM safflower would be exposed to seeds and budding plant material with expression of RNAi constructs and resulting altered fatty acid composition during harvesting. The applicant has not tested the seeds or any other tissues for altered fatty acid content driven by the gene silencing constructs.

120. People involved in the breeding, cultivating, harvesting, transporting and processing of the GM safflower may be exposed to proteins expressed through contact with the GMOs, including direct contact with GM plant material or via inhalation of pollen. This would be expected to primarily occur at the trial site but could also occur anywhere the GM seeds are transported or used. The proposed limits and controls of the trial would minimise the likelihood that people or other organisms would be exposed to GM plant material. The GM safflower is not proposed for use in commercial human food, and therefore, people are unlikely to be exposed to the introduced genetic elements or their products as a result of consuming GM safflower seed. The applicant proposes that GM safflower will only be handled by trained and authorised staff.

121. The applicant proposes human sensory testing of ingredients isolated from GM safflower seed to assess the taste, smell and texture of the oil products and protein concentrates isolated from the seed. Although the products are not intended to be swallowed during testing, some ingestion of small amounts may occur. People participating in sensory evaluations could also be exposed to products enriched in β-casein fusion proteins and altered fatty acid profiles by dermal contact, contact with mucous membranes or inhalation.

122. The GM safflower is not proposed to be used in commercial animal feed. However, animals, including birds, rodents and invertebrates, may be exposed directly to GM safflower plants at the trial sites through direct contact with plant material (e.g. through grazing or seed predation). Due to the spiny nature and unpalatability of the plant, large animals generally do not graze safflower. The applicant also proposes the use of rodent baits to reduce the number of rodents at trial sites. These measures would restrict the number of desirable animals exposed to the GM plants.

Potential harm

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

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

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).

Potential harm related to the β-casein fusion protein

125. As discussed in Chapter 1, Section 4.3, the introduced fusion proteins and genetic constructs have not been assessed for toxicity and allergenicity by any authorities or animal feeding studies. However, both caseins and the second fusion protein constituent are known food allergens. Allergy to milk proteins is most common in young children and decreases rapidly with age (Jensen et al., 2022). Allergenicity to casein proteins occurs via ingestion of the proteins. The GM safflower seeds containing β-casein fusion proteins are not intended for human consumption and, as noted in paragraph 121, only negligible levels of ingestion are likely during sensory assessment. Furthermore, allergic reactions to casein proteins via dermal contact are rare, and often require a damaged skin barrier to cause an immune response (Jensen et al., 2022). In addition, the molecular weight of a compound must be less than 500 Da in size to penetrate intact skin (Bos and Meinardi, 2000). Bioinformatic analysis of the amino acid sequence for the endogenous *CSN2* protein from which the BCN2 and BCN3 sequences are derived indicate a molecular weight (independent of the fusion protein) of 18.62 kDa, which is too large to penetrate the skin. As noted in paragraph 120, people working on the trial site or in the vicinity of the trial site could inhale airborne pollen during flowering of the GM safflower. This may lead to an allergic reaction in people allergic to safflower or any of the components of the β-casein fusion protein driven by the leaky promoter if expression in anthers results in expression of the proteins in pollen.

126. There are very few studies about toxicity of casein proteins in the literature and no reports related to humans. Some reports indicate casein tolerance in laboratory animals. An early study suggested it is almost impossible to administer lethal amounts of casein orally to rats (Boyd et al., 1967). A high-casein diet was also shown to attenuate renal injury and inflammation in rats without adverse health effects (Shimada et al., 2020). Conversely, another study demonstrated that consumption of a high-casein diet for a 4-week period resulted in a twofold increase in colonic DNA damage in rats (Toden et al., 2005). However, it is unclear whether β-casein specifically is responsible for these genotoxic effects. Further, it is unlikely that rodents will consume GM safflower seed at levels sufficient to mimic the amount of casein protein consumed in this study, and the applicant is proposing to implement rodent control measures at trial sites.

127. While studies have demonstrated allergenicity of milk proteins and caseins more broadly, no scientific evidence was identified to indicate allergenicity or toxicity of β-casein specifically in humans or animals. The genetic constructs proposed for insertion into GM safflower are derived from the sequence for the A2 variant of β-casein, which is a common component of cow's milk consumed by humans and is not associated with gastrointestinal intolerance linked to the A1 variant.

128. Further information about the β-casein fusion protein relevant to consideration of this risk scenario has been declared CCI by the Regulator. Under section 185 of the Act, the CCI has been made available to the prescribed agencies and experts that are consulted on the RARMP for this application.

Potential harm related to RNAi gene silencing constructs for SAD and FAD2

129. The introduced RNAi gene silencing constructs for *SAD* and *FAD2* could lead to production of substances that are potentially toxic or allergenic for people or toxic for other organisms.

130. 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 i.e. the introduction of the silencing constructs does not lead to expression of a novel protein. All known allergens are proteins, so there is no direct pathway leading to production of a toxin or allergen for this modification.

131. Hairpin RNA transcribed from the silencing constructs is processed into siRNAs. siRNAs fall under a general category of small RNAs (sRNAs) which also includes micro RNAs (miRNAs). siRNAs and miRNAs are

found in commonly consumed plant and animal-based foods, with several sRNAs with perfect complementarity to the human or animal genome identified in widely consumed crops, including soybean, corn and rice (Ivashuta et al., 2009). For example, corn specifically contains sRNAs complementary to 450-2300 RNA transcripts in rats, mice and humans (Petrick et al., 2016).

132. RNAi technology has been used to develop a number of GM crops, including alfalfa, potato and apple, that have been approved for use as food, feed or cultivation in a number of countries (Baylis, 2017; FSANZ, 2016). 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. The use of RNAi technology in GM safflower has been previously assessed and approved by the Regulator as part the commercial release of high oleic safflower under licence DIR 158. There are no known reports of adverse effects from the release of these GM crops.

133. It is possible that siRNAs produced in GM safflower lines could modulate expression of human or animal genes, with unknown physiological effects. 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% (Zhang et al., 2011). However, the quantity of rice fed to the mice in this study was equivalent to a human eating approximately 33 kg/day of cooked rice, and the reported effect on the mouse gene was transient, ceasing when rice was no longer included in the diet. A review paper examining data from dietary intake of sRNAs in mammals determined that dietary material does not contain sufficient sRNA to allow the uptake of biologically significant levels in mammals (Chan and Snow, 2017). Further, the applicant proposes GM safflower seeds will not be used for commercial human food or animal feed. Considering all of this information, while it is theoretically possible that the siRNAs produced by the inserted *CtSAD* and *CtFAD2.2* silencing constructs could induce off-target effects in humans or animals if consumed, it is highly unlikely that any harmful off-target silencing would occur, especially from inadvertent ingestion of the GM safflower by a person or animal.

134. There are several biological barriers which prevent a consumed siRNA initiating the RNAi pathway in a human or animal. Nuclease enzymes present in the saliva and digestive tract would denature ingested siRNAs (Huang et al., 2018). Absorption by the intestine would also require the siRNA to cross a series of cellular membranes and lipid bilayers which are often impermeable to RNAs (Petrick et al., 2013). Therefore, it is unlikely that siRNAs produced from the gene silencing constructs introduced into the GM safflower would, if ingested, cause toxicity to humans or animals.

135. 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 demonstrated to silence multiple genes in *Caenorhabditis elegans* (Timmons et al., 2001; Timmons and Fire, 1998). A number of studies have shown species-dependant variability in the ability of invertebrates to take up dietary sRNAs from different dietary sources (Chan and Snow, 2017; Dowling et al., 2016). Lepidopteran insects have low RNAi efficiency, while Orthoptera, Coleoptera and Hemiptera are more sensitive to RNAi (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 degradation of double-stranded RNA (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.

136. In a review of the scientific literature examining RNAi mechanisms in assessing biosafety risks, FSANZ concluded that the weight of scientific evidence published was not sufficient to suggest that small dsRNAs in foods are likely to have adverse consequences for humans (FSANZ, 2013b). Furthermore, the current case-by-case approach to GM food safety assessment is sufficiently broad and flexible to address GM food safety (FSANZ, 2013b).

137. The 2 genes targeted by the RNAi gene silencing construct are *SAD* and *FAD2*. These genes, described in Chapter 1 Section 4.1, are fatty acid desaturase genes. Suppression of *SAD* leads to increased stearic acid and decreased oleic acid, while suppression of *FAD2* leads to increased oleic acid and decreased linoleic

acid. Stearic acid is a saturated fatty acid found in many animal and vegetable fats and is commonly used in cosmetics. Prolonged high intake of dietary stearic acid is associated with increased cardiovascular disease risk (Hunter et al., 2010). Oleic acid is a monounsaturated fatty acid and is a major constituent of vegetable oils (such as olive oil, canola oil, sunflower oil and peanut oil) and animal fats (such as beef tallow, pork lard and poultry fat). Oleic acid is the principal fatty acid in the Western diet and is not considered toxic or allergenic (Arab, 2003). There is no reasonable expectation that the RNAi constructs introduced in the GM safflower will lead to increased toxicity or allergenicity.

138. A bioinformatic alignment analysis of the safflower *SAD* and *FAD2* sequences revealed animals do not have genes orthologous to *SAD* and *FAD2*. Therefore, even if GM safflower seed expressing one of the gene silencing constructs were ingested, there would be no complementary endogenous gene transcripts to silence. Further, as described above, the siRNAs would need to be produced at high levels in GM safflower, a large quantity of the seed would need to be consumed, and many biological barriers exist preventing the siRNAs from being taken up by cells.

Conclusion

139. Risk scenario 1 is not identified as a substantive risk because the GM plant material would not be used as commercial human food and animal feed, the GM dairy fusion proteins are not expected to be toxic, and the proteins encoded by the introduced genes are too large to penetrate an intact skin barrier and cause allergenicity via dermal contact. The gene silencing fragments do not encode proteins and the altered fatty acid profiles as a result of their expression are not associated with toxic or allergenic reactions and off-target effects of the constructs are highly unlikely. Further, the limits and controls of the field trial would restrict exposure of people and desirable animals to the GM plants. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk source	Introduced genes for dairy protein production and altered fat composition
Causal pathway	⬇ Cultivation of GM safflower at trial sites ⬇ Persistence of GM safflower seed at trial sites or dispersal of GM seed outside trial limits ⬇ Establishment of populations of volunteer GM plants expressing the introduced genes in the environment ⬇
Potential harm	Increased toxicity or allergenicity for people OR Increased toxicity to desirable animals OR Reduced establishment or yield of desirable plants

Risk source

140. The source of potential harm for this postulated risk scenario is the introduced genes for dairy protein production and altered fat composition in GM safflower plants.

Causal pathway

141. The GM safflower would be grown at the trial sites. GM seeds could be physically dispersed outside the trial sites by human activity, animal activity, wind or water. GM seeds could also persist on trial sites after completion of the trial. These GM seeds could grow in the environment and establish populations of volunteer GM plants.

142. Viable GM safflower seeds could be dispersed outside the trial sites by human activity, such as transport of seeds and movement of agricultural machinery. Dispersal of seeds by people dealing with the GMOs would be minimised by cleaning of all equipment prior to removal from the trial sites. All GM plant material would be transported in accordance with the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs.

143. Dispersal of viable seed could occur via animal activity. Safflower seeds lack seed dispersal characteristics which can contribute to seed dispersal via animal fur or feathers, but seeds could be transported externally on soil attached to feet or legs of animals.

144. Dispersal of seed could also occur via endozoochory (dispersal through ingestion and excretion of seeds). 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, 2017). 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). The researchers also mentioned birds that hoard or cache seeds such as jays, crows and ravens, as potential transport vectors of safflower seeds. Results may differ for Australian bird species such as galahs, corellas or bush turkeys. It is unknown whether Australian birds carry safflower seeds away for later consumption.

145. 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. However, the applicant proposes to till the trial sites post-harvest, which should bury the GM seeds. A 10 m monitoring zone around the trial sites is proposed that would be monitored for rodents and maintained in a manner to minimise rodent activity. Rodents would be controlled if required.

146. Dispersal of viable seed could occur through extreme weather events such as flooding or high winds. 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. Trial sites would be located at least 50 m away from natural waterways to minimise seed dispersal in the event of flooding. Seeds dispersed by flooding would be unlikely to survive and establish, as safflower is susceptible to fungal diseases in wet soil (GRDC, 2017).

147. During harvest of safflower, a small percentage of the GM seeds are expected to be lost and to remain on the trial sites. Typical safflower seed losses during harvest are 3-4% (GRDC, 2017) and the viability of these seed may range between 26-84% (McPherson et al. 2009b). Most of these seeds would germinate soon after harvest due to the low dormancy of safflower seeds (see Chapter 1, Section 3). In a Canadian study, safflower seed did not persist beyond 2 years at the surface or one year when buried (McPherson et al. 2009b). Preliminary data from trials conducted under DIR 121 (GM safflower with increased oleic acid) suggests that seed lost at harvest germinated within the first 2 months post-harvest with no further volunteers observed over the following 7 months despite conditions conducive for germination. Further, GM safflower seeds grown in the greenhouse under DIR 121 were reported to germinate and establish at the same rate as non-GM comparators. The applicant has indicated observations of the GM safflower grown in glasshouse conditions were comparable to non-GM safflower in their growth patterns, morphology, germination rates, time to flowering, plant height, seed count per plant and seed oil content. It is not expected that the production of β-casein fusion proteins in safflower would affect seed yield, viability or germination, and no information was found in the literature to suggest casein production in plants would have such effects. Likewise, while the fatty acid composition is altered, the total fatty acid content of seeds, and thus their stored energy content, is expected to remain the same, and is not expected to alter safflower seed yield, viability or germination. The only relevant study from a literature review demonstrated that cotton (*Gossypium hirsutum*) seeds with higher proportions of unsaturated fatty acids had the same mean germination time as seeds from conventional cotton cultivars (Dhaliwal et al., 2024). It is not expected that the genetic modifications would affect seed dormancy or the ability of the GM safflower to persist in the environment, or to survive the control measures being proposed, such as tilling

or irrigating the trial sites and destroying any volunteers found during post-harvest monitoring. However, this is an area of uncertainty.

148. Most sites will be used to produce one crop per year; however the applicant has indicated that GM safflower may be planted as both a summer and winter crop where soil and climate conditions are suitable. This would not impact the risk of dispersal from trial sites as the overall limits to the number of sites planted and the total area planted applies and the same controls apply to all planting sites.

Potential harm

149. If the GM safflower entered the Australian environment, the potential harms are increased toxicity or allergenicity to people, increased toxicity to desirable animals, or reduced establishment or yield of desirable plants.

150. As discussed in Risk scenario 1, no substantive risk was identified for increased toxicity or allergenicity of the GM safflower for people or increased toxicity to desirable animals. Although the β-casein fusion protein constituents may be associated with allergenicity, the GM plant material would not be used as commercial human food and animal feed, and the proteins encoded by the introduced genes are too large to penetrate an intact skin barrier to cause allergenicity via dermal contact. The gene silencing fragments do not encode proteins and the altered fatty acid profiles as a result of their expression are not associated with toxic or allergenic reactions. The limits and controls of the field trial would further restrict exposure to the GM safflower and any associated toxicity or allergenicity.

151. Populations of volunteer GM safflower could reduce establishment or yield of desirable plants. GM volunteers could directly compete with agricultural crops, pastures or native vegetation. GM volunteers could also reduce yield of commercial safflower crops by providing a reservoir for pathogens or pests. As discussed in Chapter 1, Section 3, safflower is naturalised throughout Australia, and is a minor weed not sufficiently problematic to warrant control in agricultural ecosystems (Groves et al., 2003). Safflower plants are susceptible to a wide range of herbicides as well as physical weed management practices, which are used to control volunteer populations (GRDC, 2017).

152. Anecdotal information from safflower farmers (GRDC, 2017) and weed risk assessment experts (personal communication, Stephen Johnson 2014) in Australia indicate that safflower is not a significant weed in natural ecosystems. Wild safflower is considered a minor weed that primarily establishes on disturbed ground (Groves et al., 2003). Safflower also lacks weedy qualities, including seed dormancy, high seed output, high seed dispersal, long-distance seed dispersal, seed shattering, persistent seed banks, and rapid growth to flowering (OECD, 2022). Given its poorly competitive nature and slow growth rate, safflower has limited capacity to invade in undisturbed natural areas (OGTR, 2019).

153. The genetic modifications are not expected to affect the susceptibility of GM volunteers to standard weed management measures. Although some of the GM safflower lines will contain the *bar* gene and be tolerant to glufosinate herbicide, as discussed in Section 5.3, glufosinate herbicide will not be applied to the GM safflower plants as part of this trial and other methods are available to manage GM safflower volunteers.

154. No phenotypic changes were observed between GM safflower and non-GM safflower grown under glasshouse conditions, and a standard condition of a licence for a field trial would be that the applicant immediately notify the OGTR of any unintended effects, including changes to weediness characteristics or seed dormancy. In the unlikely event of GM safflower plants establishing themselves beyond trial limits, the introduced traits 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.

Conclusion

155. Risk scenario 2 is not identified as a substantive risk because the proposed limits and controls of the field trial would minimise dispersal or persistence of GM seeds, the GM safflower is susceptible to standard weed management measures, and the genetic modifications are not expected to increase the overall

weediness or competitiveness of the GM safflower with other plants. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk scenario 3

Risk source	Introduced genes for dairy protein production and altered fat composition
Causal pathway	⬇ Cultivation of GM safflower at trial sites ⬇ Pollen from GM plants dispersed outside the trial sites ⬇ Outcrossing with sexually compatible plants ⬇ Establishment of populations of hybrid GM plants expressing the introduced genes in the environment ⬇
Potential harm	Increased toxicity or allergenicity for people OR Increased toxicity to desirable animals OR Reduced establishment or yield of desirable plants

Risk source

156. The source of potential harm for this postulated risk scenario is the introduced genes for dairy protein production and altered fat composition in GM safflower plants.

Causal pathway

157. The GM safflower would be grown at the trial sites. Pollen from the GM plants could be transported out of the trial sites by wind or insect vectors and fertilise sexually compatible plants. Hybrid seeds containing the introduced genes could be harvested by farmers or could grow as volunteers.

158. As discussed in Chapter 1, Section 5.4, there are 4 weedy *Carthamus* species that may be present in Australia: *C. lanatus, C. leucocaulos*, *C. dentatus* and *C. glaucus*. GM safflower could theoretically cross-pollinate plants from other *Carthamus* species at low levels if these weedy species were present in close proximity to the trial sites and flowered synchronously. In the highly unlikely event that hybrids between GM safflower and a related *Carthamus* weedy species occurred, they would be annuals like all *Carthamus* species and are likely to be sterile (Mayerhofer et al. 2011). Therefore, the hybrids would only be transient weeds in the immediate environs of the trial sites and would not lead to long-term transfer of the introduced genetic elements into weedy *Carthamus* species populations. Interspecific hybridisation between safflower and other species of the *Carthamus* genus present in Australia is difficult due to various cytogenetic barriers (e.g. varying chromosome number) and is highly unlikely to occur naturally as crosses have only been obtained under experimental conditions.

159. Given safflower is predominantly self-pollinating, and wind plays only 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, 2019). Field trials in a number of countries (Claassen, 1950; Kadam and Patankar, 1942; McPherson et al., 2009a; Nabloussi et al., 2013; Rudolphi et al., 2008; Velasco et al., 2012) have shown that cross-pollination frequencies and distances vary with cultivars and external factors such as climate or presence of pollinators.

160. As discussed in Chapter 1, Section 5.2, outcrossing is only observed at very low rates between safflower plants growing in close proximity. While honeybees foraging at long distance have been

documented in safflower (Gary et al., 1977), 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. This author indicates that honeybees often show fidelity to a particular feeding site, thus limiting field-to-field gene flow. Bumblebees are reported to be more effective at field-to-field pollination of safflower than honeybees (Cresswell 2010), however bumblebees are not found in mainland Australia. However, in the North Hemisphere studies, bumblebees represent less than 10% of insect visitors to safflower fields and in some studies bumblebees were not found to be present in safflower fields (Cresswell 1999; Cresswell 2000).

161. Once dispersed, pollen grains need to compete with the floret's own pollen to result in outcrossing. In safflower, 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), thus favouring self-pollination. Moreover, pollen carryover is low, with transported safflower pollen reported to only be moved to the next visited floret (Cresswell et al., 2002).

162. It is possible that volunteer plants could grow in the trial sites from residual seed from the GM safflower or from a non-GM safflower crop planted in the previous growing season. This would allow pollen flow from the GMOs growing at the trial sites to the volunteer safflower plants. The applicant proposes to prevent this by implementing control measures including promoting germination by post-harvest tillage and irrigation, and monitoring of the trial sites and destruction of any volunteer safflower prior to flowering. The effectiveness of these control measures is discussed in Chapter 3.

163. It is not expected that the intended modifications would change the pollination characteristics of the GM safflower to increase the likelihood of pollination of non-GM safflower or related species.

164. The proposed limits and controls of the trial would minimise the likelihood of pollen flow including isolation distances between trial sites and non-GM safflower crops, monitoring for and destruction of volunteers (including any hybrids) and related species during the trial and post-harvest. The effectiveness of these control measures is discussed in Chapter 3.

Potential harm

165. The potential harms from this risk scenario are adverse health effects in people and/or nontarget organisms, or reduced establishment or yield of desirable plants.

166. As discussed in Risk scenario 1, no substantive risk was identified for increased toxicity or allergenicity of the GM safflower for people or increased toxicity to desirable animals. Similarly, in the rare event of outcrossing between the GM safflower and sexually compatible plants, the proteins encoded by the introduced genes are too large to penetrate an intact skin barrier to cause allergenicity via dermal contact. Likewise, the gene silencing fragments will not encode proteins and altered fatty acid profiles as a result of their expression will not be associated with toxic or allergenic reactions in hybrid plants.

167. As discussed in Risk scenario 2, the GM safflower is not likely to be weedier or more competitive than non-GM safflower. Similarly, in hybrids between the GM plants and sexually compatible plants, the genetic modifications are not expected to confer an overall increased ability to compete with other plants.

Conclusion

168. Risk scenario 3 is not identified as a substantive risk due to the limited ability of safflower pollen to be dispersed over long distances, hybrids would be produced at low frequency and are likely to be sterile, and the expected lack of weediness in hybrid plants between the GM safflower and sexually compatible plants. Further, the proposed limits and controls are designed to minimise pollen dispersal. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

2.4.4 Risk scenario 4

Risk source	Introduced genes for dairy protein production and altered fat composition
Causal pathway	⬇ Cultivation of GM safflower at trial sites ⬇ Consumption of GM safflower seed by pest animals ⬇ Increased fitness of pest animals ⬇
Potential harm	Reduced establishment or yield of desirable plants OR Reduced biodiversity

Risk source

169. The source of potential harm for this postulated risk scenario is the introduced genes for dairy protein production and altered fat composition in GM safflower plants.

Causal pathway

170. The GM safflower would be grown at the trial sites. The GM safflower produces seeds with dairy protein production and altered fat composition.

171. Pest animals, such as rodents, large mammalian species, or birds may ingest the GM safflower seed at the trial site and may have a fitness advantage as a result of consuming the GM safflower seeds. Populations of these pests may then increase as a consequence of this increased fitness.

172. As discussed in Risk scenario 2, small birds and cockatoos may consume safflower seeds. Access to GM safflower seeds by rodents is primarily limited to fallen seed, as seeds are held in the plant capitulum. Larger pest species such as pigs or kangaroos are deterred from grazing safflower by its spines and unpalatability (GRDC, 2017), and therefore are unlikely to be exposed to the GM safflower seeds.

173. If animals do consume GM safflower seeds, it is likely that the seed will only make up a subset of the animal's overall diet, and the GM seed will only be available for a short period of time before harvest and until any seed on the soil surface is buried by tilling.

Potential harm

174. If pests consuming the GM safflower seeds with expression of β-casein fusion proteins and with an altered fatty acid profile had an advantage over those which did not consume the GM safflower seeds, and populations increased to a greater extent than would be expected otherwise, they may have a greater negative effect on native or other desirable plants. This could reduce the establishment or yield of these plants, causing damage to other crops in agricultural areas or to native vegetation. They might also increase competition for desirable animals and reduce biodiversity.

175. While there is a body of literature supporting caseins as a source of amino acids and their utility in muscle growth in humans, there is limited evidence to show similar growth benefits in animals consuming casein proteins. A study in which dairy cows were provided 300 g casein per day for 2 weeks via infusion, found that a casein-infused diet increased amino acid concentrations and milk production compared to cows fed grass silage alone (Vanhatalo et al., 2003). Another study in malnourished rats determined that re-feeding with a casein-infused diet increased bone strength and body weight catch-up compared to a whey-infused diet, but was insufficient to return the rats to a normal body weight (Masarwi et al., 2016). However, no studies were found to suggest a casein-infused diet leads to enhanced fitness or competitiveness of animals.

176. Birds may consume the GM β-casein-containing safflower seeds, however any increased fitness advantage to birds consuming the seeds is expected to be very minor, and will not be inherited by subsequent generations.

177. Some GM safflower lines proposed for release have been modified to downregulate *SAD*, expected to produce increased levels of stearic acid with a corresponding decrease in levels of oleic acid. As discussed in Chapter 1, Section 4.3, increased intake of stearic acid is associated with increased risk of cardiovascular disease in humans (Hunter et al., 2010). If GM safflower seed high in stearic acid is consumed by pest species, it is unlikely to confer enhanced fitness or competitiveness, however, this is an area of uncertainty.

178. Some GM safflower lines proposed for release have been modified to downregulate *FAD2*, expected to produce increased levels of oleic acid and decreased levels of linoleic acid. Increased oleic acid intake has been associated with health benefits in humans (Calder, 2015; Sales-Campos et al., 2013). However, it is unclear if these health benefits would provide increased fitness for 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). However, a more recent study of mice fed a diet high in oleic acid for 4 weeks found dietary oleic acid intake improved running endurance and altered composition of muscle fibres (Komiya et al., 2024).

179. For birds, a study of Japanese quail hens showed that palmitic acid played a more important role in reproductive performance as compared to oleic and linoleic acid (Vilchez et al., 1992). A study of broiler chickens fed a diet of high-oleic acid sunflower seeds did not indicate significant differences in digestive organ size or bird performance compared to chickens fed a palm oil control (Viveros et al., 2009).

180. If pest insects consuming high oleic acid safflower became more competitive as a result of high oleic acid diets, 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, 2017). Therefore, the chance for pest insects to access increased oleic acid in the GM safflower seed is low.

181. If enhanced fitness occurred in pest animals that consumed the GM safflower seed producing β-casein and altered fatty acid profiles, the improvement would be expected to be minor and transient, would be isolated to the individual animal, and are unlikely to change the existing impact of known pest animals if consumed. Additionally, access to safflower seeds by rodents and large pest animals is limited due to safflower morphology and the limits and controls of the trial proposed by the applicant. The details of those control measures are discussed in Chapter 3. Thus, the increased levels of β-casein fusion proteins, stearic acid or oleic acid available in the GM safflower seeds is unlikely to provide a fitness advantage that would increase the existing impact of known pest animals.

Conclusion

182. Risk scenario 4 is not identified as a substantive risk due to the low likelihood of pest species accessing GM safflower seeds as a result of safflower morphology, the lack of evidence to suggest any fitness advantage to pest animals as a result of consuming higher levels of β-casein fusion proteins or stearic and oleic acids and the proposed limits and controls to restrict exposure of animals to the GMOs, including pest management practices. Further, GM seed consumption is unlikely to cause sustained benefit for animals. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

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

184. Uncertainty is addressed by approaches such as balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios involving uncertainty to

lead to harm. If there is residual uncertainty that is important to estimating the level of risk, the Regulator will take this uncertainty into account in making decisions.

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

186. For DIR 211, uncertainty is noted particularly in relation to:

- potential for increased toxicity to livestock/other desirable organisms or increased allergenicity to people of the GM safflower
- potential for the genetic modifications to increase plant competitiveness and survival
- potential for the genetic modifications to increase competitiveness of pest species if consumed.

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

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

Section 4 Risk evaluation

189. 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.

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

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

191. Four risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the limits and controls proposed by the applicant, and considering both the short and long term, none of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 6 and include:

- limits on the size and duration of the proposed release
- suitability of controls proposed by the applicant to restrict the spread and persistence of the GM safflower plants and their genetic material
- the products of the introduced genes are not expected to be toxic
- limited ability of GM safflower pollen to disperse outside of trial limits
- limited ability of GM safflower to cross with weedy related species
- GM safflower plant material is not expected to confer increased fitness to pest species
- none of the GM plant material would enter commercial human food or animal feed.

192. Therefore, risks to the health and safety of people, or the environment, from the proposed release of the GM safflower plants into the environment are considered to be negligible. The Risk Analysis Framework (OGTR 2013), which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no additional controls are required to treat these negligible risks. Hence, the Regulator considers that the

dealings involved in this proposed release do not pose a significant risk to either people or the environment.

Chapter 3 Risk management plan

Section 1 Background

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

194. 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 can be managed in a way that protects the health and safety of people and the environment.

195. All licences are subject to 3 conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: section 64 requires the licence holder to provide access to premises to OGTR inspectors and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder must also be reported to the Regulator.

196. 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

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

Section 3 General risk management

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

3.1 Limits and controls on the release

199. Sections 2.1 and 2.2 of Chapter 1 list the limits and controls proposed by the applicant. Many of these are discussed in the four risk scenarios considered in Chapter 2. The appropriateness of these limits and controls is considered further in the following sections.

3.1.1 Consideration of limits proposed by the applicant

200. The applicant proposes that the field trial would take place between May 2025 and December 2029. This would limit the duration of the trial to 4 years and 8 months. The GM safflower would be grown at a maximum of 2 sites per year in 2025, 5 in 2026, 10 in 2027, 15 in 2028 and 20 in 2029. Across all sites, GM

safflower is proposed to be grown on planting areas up to a combined total area of 1 ha in 2025, 5 ha in 2026, 50 ha in 2027, 225 ha in 2028, and 700 ha in 2029 – a total of 981 ha over the duration of the licence. The size and short duration of the trial would restrict the exposure of people and desirable animals to the GMOs (Risk scenario 1). The applicant proposes up to 15 ha per site in 2028 and 35 ha per site in 2029. However, monitoring such a large area could be challenging and, at this stage, the applicant does not have experience monitoring large sites for GM field trials. Therefore, it is considered appropriate to limit the maximum combined planting area for each site to 5 ha in 2028 and 2029. This would ensure effective monitoring for volunteer GM safflower on each site.

201. The applicant proposes that only authorised and trained people would be permitted to deal with the GMOs. Standard licence conditions included in the licence state that only people authorised by the licence holder are covered by the licence and permitted to deal with the GMOs. In addition, the licence holder must inform all people dealing with the GMOs of relevant licence conditions. These measures would ensure that the field trial is conducted in accordance with the specified limits and controls (important for all risk scenarios).

3.1.2 Consideration of proposed controls regarding exposure to the GMOs

202. The applicant proposes to grow both GM safflower and non-GM safflower in the trial sites. The licence limits the plants that can be intentionally grown in the planting area to the GMOs, non-GM safflower, and any plants approved in writing by the Regulator. As non-GM safflower may be mingled with or fertilised by GM safflower, a standard licence condition has been imposed requiring non-GM safflower plants grown in a trial site to be treated as if they are GMOs. This measure manages the dispersal or persistence of GM seed (Risk scenario 2).

203. The applicant proposes that GM plants or products from the GM plants would not be used in commercial human food or animal feed, and this requirement has been included in the licence. This condition would maintain the risk context by restricting the exposure of people and desirable animals to the GMOs via consumption (Risk scenario 1) and would also minimise dispersal of the GMOs by livestock or during transport or processing for human food or animal feed use (Risk scenario 2).

204. Any human sensory testing must be approved by a Human Research Ethics Committee (HREC) in accordance with the National Statement on Ethical Conduct in Human Research. This condition would maintain the risk context by ensuring exposure of people to products derived from the GM safflower would be conducted under oversight of a HREC. This ensures (among other considerations) that those conducting sensory testing would be required to consider risks and benefits of the research and appropriate exclusion criteria for participants. It would also ensure that participants are informed of risks, including potential allergenicity, so that they can provide informed consent prior to testing.

205. People who are allergic to β-casein or the other potentially allergenic protein constituent of the fusion protein could have an allergic reaction to GM safflower as a result of the genetic modification. The applicant has proposed to restrict trial site access to authorised personnel. An additional licence condition is included to not engage personnel with a known allergy to the proteins expressed as a result of modifications in the GM safflower to conduct dealings that may expose them to the GM safflower. People working with the GM safflower could be exposed to potentially allergenic β-casein fusion proteins via contact with material from the GM safflower seeds or via inhalation of pollen if the fusion protein is expressed in pollen as a result of leakiness of the promoters used to drive expression (Section 2.4.1). However, allergic reactions to casein proteins via dermal contact is rare and both introduced proteins are too large to penetrate the skin to cause allergenicity (Section 2.4.1). Safflower pollen is not carried by wind beyond 1 m (Claassen, 1950), so even if the proteins are expressed in pollen, exposure of people at the trial site would be minimal and exposure of people outside the trial site is highly unlikely. Due to the limited scale of the proposed trial, only a limited number of people would be exposed to the GM safflower, but it is possible that one or more of those people could have a known allergy to milk proteins or to the other fusion protein constituent. Consequences of allergic reactions to these proteins can be severe, including anaphylaxis. Therefore, it is considered that the imposed condition is appropriate to protect people with known allergies to these proteins from contact with GM safflower seed or pollen (Risk scenario 1).

206. The applicant has proposed fencing around trial sites to restrict access by large animals. This would limit exposure to GM safflower to large animals through direct contact with plant material (e.g. through grazing). However, as discussed in Risk scenario 1, it is unlikely that safflower will be grazed by large animals due to its spiny nature and unpalatability, and the licence does not allow the GM safflower to be used as animal feed. Furthermore, if consumed, potential harm to desirable animals from the introduced genetic elements is expected to be minimal. Therefore, the licence does not impose the use of fencing at trial sites.

3.1.3 Consideration of proposed controls regarding pollen flow from the GMOs

207. The applicant has proposed that trial sites may contain one or more GM safflower planting areas (Figure 2, Chapter 1). Each planting area is proposed to be surrounded by a 10 m monitoring zone and a 50 m inspection zone, in which safflower and related species would be controlled, and then surrounded by a 140 m isolation zone in which no safflower or related species would be intentionally grown.

208. The applicant proposes to maintain the 10 m monitoring zone in a manner appropriate to allow the identification of volunteer safflower and related species, and in a manner to minimise rodent activity. This would remove safflower plants or related species that may hybridise with GM safflower (Risk scenario 3), facilitate detection of GM plant material that has been dispersed during harvesting (Risk scenario 2), and avoid attracting or harbouring rodents (Risk scenarios 2 and 4). A condition has been included in the licence requiring a 10 m monitoring zone around each trial site to be maintained in a manner to minimise rodent activity. Additional licence conditions require inspection of the monitoring zone while the GMOs are growing and post-harvest for identification and destruction of volunteer plants of safflower.

209. As discussed in Chapter 1, pollen-mediated outcrossing rates from GM to non-GM safflower were 1.7% to safflower plants 3 m from donor plots, 0.01% to safflower 100 m away, and undetectable with 300 m separation (McPherson et al., 2009a). As discussed in Risk scenario 3, pollen flow from the GM safflower might occur not only to GM safflower volunteers, but also to volunteers that have grown from residual seed from previous safflower crops as viable safflower seed on the soil surface can persist up to 2 years. Therefore, separation of the GM safflower in the planting area from isolated safflower plants by 200 m (10 m monitoring zone plus 190 m inspection zone) is considered appropriate for minimising gene flow to these plants, rather than the proposed 60 m (10m monitoring zone and 50 m inspection zone). Similar to conditions imposed in DIR-131, the 190 m area to be inspected can be reduced to 50 m if no safflower crops have been grown within 200 m of the planting area in the previous 2 years or if no safflower plants were observed in this area in the previous growing season.

210. The applicant has proposed that monitoring and inspection zones would be inspected from 14 days prior to flowering until the entire planting area has completed flowering, to identify and destroy volunteer safflower or related species. Safflower requires approximately 45 days to develop from its rosette stage to flowering, but this development can be hastened in hotter or drier conditions (GRDC, 2017). Given this, during the inspection period (14 days prior to flowering to completion of flowering of all GMOs in the planting area), it is considered appropriate to inspect the monitoring zones and inspection zones for volunteers at least once every 14 days. These measures would minimise gene flow to naturalised safflower (Risk scenario 3). The licence includes a condition to impose monitoring for volunteer safflower according to these considerations.

211. The applicant proposed to isolate the GM safflower from intentionally grown safflower crops by 200 m. The Australian safflower industry is small (see Chapter 1, Section 3), thus a commercial safflower crop is unlikely to be in close proximity to a particular trial site. However, if a safflower crop was planted close by while the GM safflower is flowering, outcrossing could occur at distances greater than 200 m. International guidelines for production of basic safflower seed recommend an exclusion distance of 400 m from other safflower cultivars (OECD, 2013). The Association of Official Seed Certifying Agencies (AOSCA) (2012) recommends an isolation distance of 1320 ft (403 m) for producing certified safflower seed of all seed classes. However, at this exclusion distance there could still be very low levels of cross-pollination, and there is a lack of scientific studies addressing efficacy of exclusion distances for safflower. In these circumstances of uncertainty, it is considered appropriate to increase the exclusion distance by a safety factor. For a Canadian field trial of GM safflower in 2011, the Canadian Food Inspection Agency (see CFIA

website) required that GM safflower plants be reproductively isolated from other safflower plants by 800 m, and from safflower seed production by 1600 m. However, the GM safflower in the Canadian trial expressed a pharmaceutical compound that could have adverse effects on humans or animals if ingested, justifying a large isolation distance. In the USA, field trials of GM safflower expressing a pharmaceutical compound required an even larger isolation distance of 2 miles (3.2 km) to the nearest commercial safflower crop (USDA-APHIS 2008). APHIS has processed over 25 safflower permits since 2003 and has found no significant impacts to humans or the environment (USDA-APHIS 2008). Based on these considerations, the licence requires that GM safflower must not be grown within 600 m of a safflower crop that is not a part of the field trial, rather than within 200 m as proposed by the applicant.

212. GM safflower will be isolated from intentionally grown safflower crops by a 590 m isolation zone. The isolation zone would be inspected for any safflower crop every 35 days until the GMOs growing in the planting area have been harvested. Any identified safflower crop would be destroyed before flowering or prevented from flowering, or the GMOs in the planting area would be destroyed before flowering. As noted above, this would minimise gene flow from the GMOs to other safflower plants and sexually compatible crops which could grow from residual seed planted in the previous year (Risk scenario 3). The licence also requires that the GMOs must not be planted if a safflower crop other than those grown pursuant to this licence was planted in the isolation zone in the previous growing season. This is because there could be a high abundance of volunteers in the year immediately after planting a safflower crop, which could result in outcrossing in the isolation zone. The field trial setup is included in the licence.

213. The applicant has proposed the use of plant bagging and pollen control tents if necessary. Bagging or tenting would be used on individual safflower plants to facilitate manual breeding and to prevent contamination of genetic material of their GM breeding material by unintended pollen flow from other safflower plants. GM safflower plants would only be bagged or tented during specific periods when needed to manage pollination. Use of bags or tents is not proposed for limiting exposure of people to GM safflower pollen, or pest access to the GMOs. These risks are managed by other control measures included in the licence. Therefore, use of plant bagging and pollen control tents has not been included in the licence.

214. As discussed in Risk scenario 3, it is highly unlikely that hybrids between GM safflower and a related *Carthamus* weedy species will occur, and any hybrids are likely to be sterile and will not persist. Although the applicant proposed to inspect for related species in the monitoring and inspection zone while the GMOs are flowering, the available evidence does not support this measure. Thus, there is no requirement in the licence to inspect for related species. However, it should be noted that the requirement to maintain the monitoring zone in a manner that does not attract or harbour rodents and that allows the identification of safflower volunteers while the GMOs are growing will essentially have the effect of controlling any related species from flowering in the monitoring zone. Furthermore, many related species are controlled in agricultural settings as they are considered weeds.

3.1.4 Consideration of proposed controls regarding dispersal of the GMOs

215. The applicant proposes that any equipment used with the GMOs would be cleaned as soon as practicable and before use for any other purpose, to avoid movement of viable plant material together with equipment. Personnel and clothing will also undergo physical examination before leaving the trial site to prevent unintentional movement of GM material. The applicant would contain the GM seeds during transport and storage in accordance with the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs. These measures for the handling of GMOs would minimise exposure of people and other organisms to the GMOs (Risk scenarios 1 and 4), and dispersal of GMOs into the environment (Risk scenario 2) during transport, and have been included as licence conditions.

216. The applicant proposes to control the number of rodents present at trial sites. This will involve use of commercially available baits placed around the trial site and may involve housing baits in bait stations to protect them from weather conditions. This would limit the potential dispersal of GMOs outside the trial sites (Risk scenario 2) and restrict access of pests to the GMOs (Risk scenario 4). A condition in the licence requires that rodent control measures must be in place in planting areas from at least 7 days prior to planting the GMOs, while the GMOs are being grown, and until the planting area is cleaned. Another

licence condition requires that the monitoring zone is managed in a manner that does not attract or harbour rodents during this period.

217. As discussed in Risk scenario 2, safflower seeds may be predated by bird species. Although safflower seeds that have passed through the digestive system of several Northern Hemisphere bird species have been observed to be non-viable, it is unclear whether safflower seeds are rendered non-viable by digestive systems of Australian bird species. The applicant has not proposed use of bird control measures, however, to limit potential dispersal of GM safflower seed by birds, it is considered appropriate to minimise bird access to the GMOs by covering the planting area with bird netting, using commercial bird scarers and/or planting decoy crops such as sorghum near to the planting areas. A decoy crop was also considered as an effective measure for deterring birds from feeding on GM safflower in the field under licences DIR 121 and DIR 131. These measures would minimise bird activity at trial sites and thereby limit exposure of wildlife to the GMOs (Risk scenarios 1 and 4) and limit potential dispersal of GMOs outside the trial sites (Risk scenario 2). A condition in the licence requires that for the period from 14 days after commencement of flowering of the GMOs in a trial site until the site has been cleaned, the trial site must have one or more of these measures in place.

218. The applicant proposes that trial sites are located at least 50 m from natural waterways to minimise the likelihood of viable plant material being washed away from the trial site. This is considered appropriate and has been included as a condition in the licence. In addition, a licence condition has been imposed requiring immediate notification to the Regulator of any extreme weather conditions affecting the trial sites during the period of release. These measures would minimise dispersal of GM safflower outside the proposed trial sites by flooding (Risk scenario 2).

219. GM safflower seeds could be dispersed short distances from the trial sites during harvest activities, by seed hoarding behaviours of animals such as ants or rodents, or by strong winds or runoff after heavy rain. As described in Section 3.1.3, the planting areas would be surrounded by monitoring zones and inspection zones that are inspected while the GMOs are growing, so any volunteers growing from dispersed GM seeds during this period would be detected and destroyed. The applicant also proposes to inspect the planting areas after cleaning to destroy any volunteers. As the short-distance seed dispersal mechanisms listed above are unlikely to transport seeds further than 10 m from the trial sites, the licence requires post-harvest inspections of the planting areas and 10 m monitoring zone.

220. The licence includes additional conditions to manage short-distance dispersal of GM seeds. This includes requiring planting areas and monitoring zones to be cleaned within 14 days after harvest by a method that removes GM seeds from the soil surface. Although the applicant has not proposed cleaning of other areas, the licence requires areas where equipment has been cleaned, and any other areas where GMOs are known to have dispersed are cleaned as soon as practicable. This combination of controls would minimise short-distance dispersal of GM seeds leading to establishment of volunteer populations outside the trial sites (Risk scenario 2).

3.1.5 Consideration of proposed controls regarding persistence of the GMOs

221. After harvest of each trial site, the applicant proposes to destroy GMOs not required for further evaluation or future trials. This would involve both cleaning the trial site within 14 days after harvest in a manner that destroys any surviving GMOs and destroying any harvested GM seed that is not required for experimentation or future planting.

222. The applicant has proposed that GMOs would be destroyed by destructive analysis (e.g. ground up, hammer milled and/or roller milled), herbicide application, root cutting and shredding/mulching, uprooting, burning/incineration, light tillage to a depth of no more than 5 cm, autoclaving, or seed burial to a depth of at least 1 m. These methods are considered effective for rendering safflower plants and/or seed non-viable, and have been included in the licence. To ensure the effectiveness of destruction by seed burial, a licence condition specifies how this must be carried out, including a requirement that seeds must be wet at time of burial to encourage decomposition.

223. As discussed in Section 3.1.2, the applicant proposes to also grow non-GM safflower on the trial sites, which would be treated as if they are GMOs. Non-GM safflower in the trial site may be cross-pollinated by

the GM safflower, resulting in hybrid seeds. Therefore, it is appropriate to require non-GM safflower to be destroyed in the same manner as GM safflower, to manage persistence of the GMOs. This measure is included in the licence.

224. The applicant proposes to inspect the trial sites for a period of 24 months post-harvest and until no safflower volunteers have been identified in the area for at least the final 6 months of inspections. The applicant did not propose a frequency of post-harvest inspections. As discussed in Risk scenario 2, safflower seeds have low dormancy, with buried seeds reported to be non-viable beyond one year (McPherson et al., 2009b). Therefore, it is considered unnecessary to inspect the trial sites for 24 months. The licence requires post-harvest monitoring at least once every 35 days for at least 12 months and destruction of any volunteers identified, until no volunteers are identified for at least 6 months. This is consistent with licence conditions imposed as part of the limited and controlled release of GM safflower approved previously approved by the Regulator (DIR 131). Records must be kept of monitoring activities and findings, including number and location of volunteers, which will allow the Regulator to assess the ongoing suitability of these measures and provide additional information for future assessments.

225. The applicant also proposes that safflower volunteers identified during inspections would be destroyed prior to flowering, which would prevent GM safflower seed dispersal (Risk scenario 2) and pollen flow to non-GM plants outside the trial site (Risk scenario 3).

226. The applicant proposes to clean the trial sites and adjacent areas after harvest by light tillage to a depth of no more than 5 cm. During harvesting, plant material could be scattered into the area immediately surrounding the planting area, so there is potential for residual seed to be present in both the planting area and the monitoring zone. The applicant proposes to conduct tillage of the planting area one month post-harvest. In Risk scenario 2, it was noted that residual seed on the soil surface following harvest would be susceptible to dispersal by animal predation or transport, following extreme weather events such as strong winds, or by water runoff from heavy rainfall. Therefore, it is appropriate to require cleaning of the planting area and other areas where seed may have spread shortly after harvest to encourage seed germination. Therefore, a condition in the licence requires that GMO planting areas and their associated monitoring zones must be cleaned by removing destroying any GMOs and removing any safflower seeds from the soil surface within 14 days after harvest of the GMOs.

227. The applicant has proposed watering at the trial sites is defined as rainfall which provides sufficient soil moisture to promote germination of residual GM safflower seeds, or irrigation which provides the equivalent soil moisture. The applicant has not specified a frequency of watering events at trial sites post-harvest. However, given the low dormancy of safflower seeds, adequate post-harvest soil moisture is likely to promote germination and manage survival and persistence of viable safflower seeds in the soil (Risk scenario 2). Consistent with the previous GM safflower release under licence DIR 131, the licence includes a condition requiring any areas that have been cleaned to receive at least one watering event (either by rainfall or irrigation) in the post-harvest period during the 6-month volunteer-free period. This watering event must be conducted after tillage, to ensure conditions are conducive to germination of volunteers.

228. The combination of control measures described in this section would minimise the persistence of GM seeds leading to establishment of GM volunteer populations in the environment (Risk scenario 2).

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

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

- limit the duration of the release to the period from May 2025 to December 2029
- limit the size of the release to a maximum of 2 sites per year in 2025, 5 in 2026, 10 sites in 2027, 15 sites in 2028 and 20 sites in 2029, with planting areas up to a combined total area of 1 ha in 2025, 5 ha in 2026, 50 ha in 2027, 75 ha in 2028, and 100 ha in 2029
- limit the location of the release to nominated local government areas in NSW, Vic, WA and SA
- not allow GM plant material to be used in commercial human food or animal feed

- treat any non-GM safflower grown in planting areas like the GMOs
- not permit persons with an allergy to the proteins produced as a result of the modification, or related proteins to conduct dealings that may expose them to GM plant material
- surround planting areas with a 10 m monitoring zone and an inspection zone of at least 50 m or 190 m that are inspected at least once every 14 days while the GMOs are flowering to destroy any or safflower or sexually compatible plants
- surround the monitoring zone with a 590 m isolation zone where no safflower plants may be grown
- locate trial sites at least 50 m from any natural waterways
- implement measures to control rodents and birds within the planting areas
- transport and store the GMOs in accordance with the Regulator's guidelines
- destroy all GMOs not required for further evaluation or future trials
- clean equipment used with the GMOs before use for any other purpose
- clean the planting areas, monitoring zones, areas where equipment has been cleaned, and other areas where GMOs are known to have dispersed after harvest
- apply any measures to promote the germination of any safflower seeds that may be present in the soil after harvest, including watering and shallow tillage
- monitor each trial site at least once every 35 days for at least 12 months after harvest and until no volunteers are identified for at least 6 months, and destroy any safflower plants that emerge.

3.2 Other risk management considerations

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

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

3.2.1 Applicant suitability

231. 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.

232. Licence conditions include a requirement for Miruku to inform the Regulator of any information that would affect their suitability.

233. In addition, Miruku must have access to an Institutional Biosafety Committee (IBC) and be an accredited organisation under the Act.

3.2.2 Contingency plan

234. Miruku is required to submit a contingency plan to the Regulator before planting the GMOs. This plan would detail measures to be undertaken in the event of any unintended presence of the GM safflower outside permitted areas.

235. Before planting the GMOs, Miruku is also required to provide the Regulator with a method to reliably detect the GMOs or the presence of the genetic modifications in a recipient organism.

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

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

3.2.4 Reporting requirements

237. The licence requires the licence holder to immediately report any of the following to the Regulator:

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

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

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

3.2.5 Monitoring for compliance

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

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

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

Section 4 Issues to be addressed for future releases

242. Additional information has been identified that may be required to assess an application for a commercial release of the GM safflower, or to justify a reduction in limits and controls.

243. This includes:

- additional molecular and biochemical characterisation of the GM safflower lines, particularly with respect to expression of the introduced genes, proteins and fatty acid levels in the seeds and pollen
- biochemical characterisation of the GM safflower lines, particularly with respect to potential for allergenicity related to the introduced genetic elements
- additional phenotypic characterisation of the GM safflower lines leading to potential for increased weediness.

Section 5 Conclusions of the RARMP

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

245. However, licence conditions have been imposed to limit the release to the proposed size, location and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment, as these were important considerations in establishing the context for assessing the risks.

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Appendix A: 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[4] on the consultation RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence and were used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence. Advice received is summarised below.

Submission	Summary of issues raised	Comment
1	Agrees that the risk assessment identifies all plausible risk scenarios by which the proposed release could give rise to risks relating to the health and safety of people or the environment.	Noted.
	Agrees with the overall conclusion of the RARMP.	Noted.
	Advises that the Regulator should further consider risks associated with the potential for increased dormancy and increased weediness as a result of the fusion protein and changed fat profile.	The RARMP discusses data from trials of GM safflower with altered fat composition conducted under DIR 121, which suggest no change in germination rates between the GM and non-GM safflower (Risk scenario 2, Chapter 2). Text has been added to clarify this point. Risk scenario 2 also discusses the potential for the fusion protein to increase dormancy or weediness of the GM safflower. Text has been added to state that no reports were found in the literature to suggest production of the β-casein fusion protein could increase dormancy or weediness of the GMOs. Preliminary data from the applicant suggest no changes in growth, germination rates, time to flowering or seed count per plant between non-GM safflower and the GMOs. Text has been added discussing findings of a study about the effects of fatty acid composition in cotton seeds to support the conclusion of this risk scenario, that this altered trait in the GM safflower is unlikely to affect dispersal or persistence of GM seeds. In the unlikely event of increased dormancy or dispersal, the imposed control measures, including post-harvest monitoring for volunteers, would restrict GM seed dispersal and persistence.
	Advises that the Regulator should further consider controls in regard to cleaning of equipment and verification of the cleaning process.	The licence requires that equipment used in connection with the GMOs are cleaned after use and before use for any other purpose, which requires removal or destruction of the GMOs. The OGTR has issued many licences for dealings with GM crops, including other safflower licences, and the condition

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

Submission	Summary of issues raised	Comment
		requiring cleaning of equipment has been highly effective in preventing spread and persistence of GMOs. It is considered unlikely that verification of the cleaning process would increase the effectiveness of the cleaning condition already imposed by the licence.
	Advises that the Regulator should seek more detail about the sensory testing, and whether further controls around the testing are required.	The licence prohibits the use of GM plant material as human food, except as part of sensory testing. The licence also requires that sensory testing must be conducted under oversight by a Human Research Ethics Committee (HREC), which is required to review and approve the research proposals in accordance with the National Statement on Ethical Conduct in Human Research and therefore the risks associated with the GM products considered and managed under that framework. Some additional text about this has been added in Chapter 3 of the RARMP.
	Advises that the Regulator should reconsider the distance of the trial sites from waterways.	Safflower is resistant to shattering or lodging, is not adapted for transport by water, is sensitive to waterlogging, and seeds readily germinate in moist conditions. Thus, it is unlikely to persist if spread in this manner. Given these characteristics, a 50 m distance from waterways (in combination with not planting in flood prone areas) is considered appropriate, and is consistent with previous licences for limited and controlled release of GM safflower and other GM crops.
	Advises that the Regulator should consider clarifying what is considered flood prone.	When selecting a site for a planting area, a licence holder must consider a number of factors related to potential for flooding. This includes, but is not limited to, site flooding history, distance from waterways and topography. A prescriptive definition of flood prone is not practical for a number of reasons, as is reflected in the range of definitions across different jurisdictions and for different purposes. Additionally, current definitions may not be appropriate for future conditions, including changes resulting from climate change. Guidance is now included as a note to Condition 30 of the licence, to provide clarity for the licence holder about how 'flood prone' may be considered for a site.
2	No advice or comments on the RARMP.	Noted.
3	Based on the information provided is comfortable that the controls of the field trial would restrict exposure of people, the food chain, agricultural animals, and by extension the marketing of animals.	Noted.
	Notes the novelty of the GMO, and the precautions considered in the application are similar to those considered for herbicide resistant crops.	Noted.

Submission	Summary of issues raised	Comment
	Raises concern about the genetically engineered A2 β-casein as a food product, and stated an inability to locate discussion of A2 β-casein in the application, except for discussion of taste testing.	A2 β-casein is discussed in Chapter 1, Sections 4.1.1 and 4.3.1 of the RARMP.
	Notes that the applicant should consider product safety and safflower agronomy, including toxicity testing on mice, prior to human evaluation.	The RARMP concludes that risks associated with toxicity and allergenicity resulting from exposure to the GMOs are negligible. Human sensory testing must be conducted with oversight of an HREC (licence condition 20), which is required to review and approve the research proposals in accordance with the National Statement on Ethical Conduct in Human Research, and therefore the risks associated with the GM products are considered and managed under that framework. More information about this has been added to the RARMP (Section 3.1.2). If the applicant proposes to release products derived from the GMOs more broadly, safety and toxicity of products derived from the GM safflower will be assessed by FSANZ and commercial use of the products must be authorised by FSANZ.
	Notes marker genes are widely used in transgenesis. Their use has been assessed numerous times and risks found to be negligible.	Noted.
	Notes the potential allergenicity of β-casein proteins, and a lack of historical assessments relating to GM products containing β-casein. Notes that GM safflower in this licence application will not be used for human food or animal feed, and that volunteer plants will be controlled. Further notes that additional analyses will be required if the GMOs were to be grown for human consumption commercially.	Noted.
	Notes the relatively lower risk associated with the introduction of gene silencing constructs compared to insertion of coding gene sequences. Also notes the negligible risk associated with the altered fatty acid profile as a result of the gene silencing constructs introduced into the GM safflower.	Noted.
4	Agrees with the overall conclusion of the RARMP. Concludes that limited and controlled release of the GMO poses negligible risks to the health and safety of people or the environment under the proposed controls and conditions limiting the scale, location and duration of the release, and measures to restrict the spread	Noted.

Submission	Summary of issues raised	Comment
	and persistence of the GMOs and their genetic material in the environment.	
5	Notes that the licence will prohibit the use of GM plant material in human food or animal feed.	Noted.
6	Notes the uncertainty regarding potential impact of the GM safflower on native Australian birds, and the possibility of bird-mediated seed dispersal. Supports the licence conditions requiring control measures to limit access by birds to the GMOs.	Noted.
	Notes the potential for pollen flow and supports the expanded monitoring and isolation zones required in the licence.	Noted.
	Satisfied that the proposed trial pose negligible risk to human health and safety and the environment.	Noted.