Australian Government
Department of Health
Office of the Gene Technology Regulator

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Risk Assessment and Risk Management Plan for

DIR 169

Limited and controlled release of microalgae genetically modified for increased production of fatty acids

Applicant: The University of Queensland

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application No. DIR 169

Decision

The Gene Technology Regulator (the Regulator) has received a licence application for the intentional release of a genetically modified organism (GMO) into the environment. It qualifies as a limited and controlled release application under the *Gene Technology Act 2000* (the Act). The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed trial poses negligible risks to human health and safety and the environment, and that any risks posed by the dealings can be managed by imposing conditions on the release.

The application

Application Number	DIR 169
Project Title	Limited and controlled release of microalgae genetically modified for increased production of fatty acids[1]
Parent organism	*Nannochloropsis oceanica*
Genes responsible for the modified traits	**Introduced gene conferring increased production of fatty acids** NTE – thioesterase gene from *Nannochloropsis oceanica* **Partial deletion of genes conferring inability to use nitrate as a nitrogen source** NRT – nitrate transporter gene NR – nitrate reductase gene
Genetic modification method	Electroporation
Number of lines	Up to five GM lines
Proposed location	The University of Queensland's Pinjarra Hills campus (Centre for Solar Biotechnology pilot plant), Brisbane City, Queensland
Proposed release size	Multiple batches of GM microalgae in up to six securely covered culture vessels with a volume of up to 600 litres per vessel
Proposed period of release	Several periods up to a total of 12 months, until the end of 2023[2]
Principal purpose	To assess and optimise growth characteristics and production conditions of the GM microalgae under outdoor conditions

[1] The original title for the application was 'Limited and controlled release of *Nannochloropsis oceanica* genetically modified for increased production of fatty acids'.
[2] During consultation, UQ amended their application to extend the proposed period.

Risk assessment

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

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

Credible pathways to potential harm that were considered included exposure of people or other desirable organisms to the GM microalgae, and the potential for persistence or dispersal of the GMOs. Potential harms associated with these pathways included toxicity or allergenicity to people, toxicity to desirable animals, and environmental harms due to reduced quality of the biotic environment or reduced establishment of desirable organisms.

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

Risk management

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

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. Controls are included to prohibit the use of the GM microalgae in human food and animal feed, to minimise dispersal of the GMOs from the trial site, to transport GMOs in accordance with the Regulator's guidelines, and to destroy the GMOs at the end of the trial.

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Abbreviations

ACP	Acyl carrier protein
Act	Gene Technology Act 2000
APVMA	Australian Pesticides and Veterinary Medicines Authority
ppb	Parts per billion
DIR	Dealings involving Intentional Release
DNA	deoxyribonucleic acid
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GMO	Genetically modified organism
h	Hour(s)
L	Litre(s)
m	Metre(s)
mg	Milligram(s)
µg	Microgram(s)
NLRD	Notifiable Low Risk Dealing
NO_x	Nitrate + nitrite
NR	Nitrate reductase
NRT	Nitrate transporter
NTE	Acyl-acyl carrier protein thioesterase
OGTR	Office of the Gene Technology Regulator
PCB	Polychlorinated biphenyl
PCR	Polymerase chain reaction
Qld	Queensland
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
SARDI	South Australian Research and Development Institute
TERAs	Toxic Substances Control Act Experimental Release Applications
UQ	The University of Queensland
US EPA	United States Environmental Protection Agency
WHO	World Health Organization
WT	Wild type

Chapter 1 Risk assessment context

Section 1 Background

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

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

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

4. The Risk Analysis Framework (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The general risk criteria are considered appropriate for this application. 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. 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 information for establishing the risk assessment context for this application.

RISK ASSESSMENT CONTEXT

The GMO
Modified genes
Novel traits

Parent organism (comparator)
Origin and taxonomy
Cultivation and use
Biology

Proposed GMO dealings
Activities
Limits
Controls

Previous releases
Australian approvals
International approvals

Receiving environment
Environmental conditions: abiotic and biotic factors
Production practices
Related organisms
Similar genes and proteins

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

6. 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. One public submission was received and its consideration is summarised in Appendix B.

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

Section 2 The proposed dealings

8. The University of Queensland (UQ) proposes to release up to five cell lines of *Nannochloropsis oceanica* genetically modified for increased production of fatty acids and inability to use nitrate as a nitrogen source. The main purpose of the release is to assess and optimise growth characteristics and production conditions of the GM microalgae in Queensland's climate under outdoor conditions.

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

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

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

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

10. The release is proposed to take place over several periods of time, up to a total of 12 months, between 2019 and 2023[3]. Genetically modified (GM) *N. oceanica* would be grown on a single pilot plant site, with the microalgae cultivated in up to six culture vessels (up to 600 litre cultivation volume each; Figure 2). The site would be located at UQ's Pinjarra Hills campus in Brisbane, Queensland.

11. Only trained and authorised staff would be permitted to deal with the GM *N. oceanica*.

Figure 2 Small raceway ponds that would be used as culture vessels. These would be covered with secure lids during cultivation of the GMOs. Image supplied by applicant.

[3] In the initial stages of the application, the applicant requested the release to take place until 2022. The applicant subsequently requested an increase to the licence period to 2023 to allow extra time for approval and preparation of lids for the culture vessels.

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

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

- locating the site at least 200 m from a natural fresh waterway, approximately 800 m from brackish river water and approximately 28 km from a marine environment, on land that is not prone to flooding
- installing bunding (a lip) around the pilot facility to contain spills and prevent runoff
- securely fitting clear plastic lids to open culture vessels to limit the dispersal of GM microalgae
- modifying the GM *N. oceanica* to be incapable of using environmental nitrate as a nitrogen source to limit persistence in the natural environment
- fencing the site to restrict access by large animals, and continuing pest management for rodents and snakes
- decontaminating culture vessels and equipment to ensure that GM *N. oceanica* do not remain after harvest
- monitoring the waste water tank post-harvest for GM microalgae growth at least quarter yearly for at least 12 months, and until the last six months are free of GM *N. oceanica*
- not allowing any material from the GM microalgae to be used in the production of human food or animal feed
- transporting and storing GM microalgae in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*.

Section 3 The parent organism

13. The parent organism is *N. oceanica* Suda & Miyashita. The strain (CS-246) was isolated from Deception Bay, Qld, Australia (Fawley et al., 2015).

14. Detailed information about *N. oceanica* is contained in the reference document *The Biology of* Nannochloropsis oceanica *Suda & Miyashita* (a microalga) (OGTR, 2019), which was produced to inform the risk analysis for licence applications involving GM *N. oceanica*. The proposed dealings with the GM *N. oceanica* are evaluated against non-GM *N. oceanica* as a baseline.

15. *Nannochloropsis oceanica* is a single-celled microalga belonging to the kingdom Chromista (OGTR, 2019). There are currently five named species of *Nannochloropsis*: *N. australis, N. granulata, N. limnetica, N. oceanica* and *N. oculata* (Suda et al., 2002; Fawley et al., 2015). All species of *Nannochloropsis* are marine, except *N. limnetica*, which is found in freshwater environments.

16. *Nannochloropsis oceanica* is found in coastal waters of the Pacific Ocean, Atlantic Ocean and Indian Ocean (OGTR, 2019). In Australia, *N. oceanica* was isolated from Deception Bay, Qld, approximately 40 km from the proposed trial site (Fawley et al., 2015). *Nannochloropsis* sp. was isolated, using seawater growth medium, from the Brisbane River approximately 11 km from the proposed trial site (Lim et al., 2012).

17. *Nannochloropsis* is predominantly cultivated for use as a food source and 'green water' in aquaculture production (Al-Hoqani et al., 2017; OGTR, 2019). In Australia, the South Australian Research and Development Institute (SARDI) has cultivated microalgae, including *N. oceanica*, in outdoor raceway culture vessels and photobioreactors in Adelaide, South Australia (SARDI, 2015). *Nannochloropsis* sp. has been grown for biofuel production in a pilot facility near Karratha, WA (Murphy, 2013).

18. There is no evidence that *N. oceanica* produces allergenic substances. No allergens from organisms belonging to the kingdom Chromista are registered in the WHO/IUIS[4] Allergen Nomenclature database (WHO/IUIS Allergen Nomenclature Sub-Committee, accessed 30 July 2019). *Nannochloropsis* is also not

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

known to produce toxins. Several studies have found no toxicological effects of *N. oceanica* and related species when fed to rodents or brine shrimp, or applied to fibroblasts (OGTR, 2019 and references therein). Digestibility of *N. oceanica* is lower than fishmeal. *Nannochloropsis* and other microalgae may accumulate toxicants, such as heavy metals, from the environment (OGTR, 2019).

19. No algal blooms caused by *N. oceanica* have been reported to date; however, other species of *Nannochloropsis* have been implicated in marine and freshwater blooms (OGTR, 2019). These blooms are not recorded in the IODE Harmful Algal Event Database (accessed 30 July 2019).

20. *Nannochloropsis oceanica* is able to be controlled using bleach, as well as with various herbicides and antibiotics (OGTR, 2019).

21. *Nannochloropsis oceanica* is unable to reproduce sexually, due to a lack of genes associated with meiosis (Pan et al., 2011). Reproduction occurs via mitosis, with average doubling times ranging from 14 to 41 hours, depending on culture conditions (OGTR, 2019 and references therein). Survival structures have not been identified in *Nannochloropsis*.

22. Microalgae are readily dispersed via aerosol formation, entering the atmosphere by processes such as bubbles bursting in sea foam or aerated aquariums (Schlichting Jr, 1974). Their small size allows microalgae to be transported potentially over thousands of kilometres, remaining airborne for many days, before being deposited by particle settling or by removal via precipitation (Wilkinson et al., 2012; Tesson et al., 2016). *Nannochloropsis* from the local environment was detected in a dispersal trap in experiments by Szyjka et al. (2017, Fig. S3B). The survival of aerially dispersed microorganisms depends on their ability to both withstand the abiotic stresses encountered during transit and to become established in the environment in which they are deposited. Microalgae can also be dispersed via water and by animals, including birds, insects and humans (OGTR, 2019).

23. Humans, animals and other organisms can come into contact with *N. oceanica* in different ways. Marine organisms and seabirds are exposed to *N. oceanica* through coexistence in the same habitat. Humans and land-based animals can come into contact with *N. oceanica* via exposure to marine or brackish waters, or via exposure to *N. oceanica* in cultivation systems. Exposure can occur via the skin, by inhalation or by ingestion. Sensitisation to microalgae has been reported, particularly to species that are common in the air of human environments (OGTR, 2019 and references therein).

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

4.1 Introduction to the GMOs

24. The applicant proposes to cultivate up to five cell lines of GM *N. oceanica*.

25. The GM *N. oceanica* lines were transformed using electroporation (Kilian et al., 2011). The introduced gene integrated into the haploid *N. oceanica* genome via homologous recombination (Figure 3). The insertion site was selected to knock out two genes involved in nitrate use: a nitrate transporter gene and a nitrate reductase gene. Targeted gene knockout and gene replacement by homologous recombination is a technique commonly used for transformation of bacteria and yeast (Weeks, 2011).

26. The source organism for the introduced gene, targeting sequence and regulatory sequences is a strain of *N. oceanica* (NIES-2145 from the National Institute for Environmental Studies, Tsukuba, Japan). Previously, strain NIES-2145 was identified as *N. oculata*; however, the current classification is *N. oceanica* (cf. Ozaki (2016) and Algae Resource Database, accessed 22 July 2019).

27. Genetic elements modified in the GM *N. oceanica* strain CS-246 lines are shown in Table 1. This includes short regulatory sequences that control expression of the genes.

28. A chloroplast transit sequence of violaxanthin/chlorophyll a binding protein gene (VCP1SP) is inserted ahead of the NTE gene (Ozaki, 2016). Many proteins, such as acyl-ACP thioesterase, that carry out processes in the chloroplast are encoded by nuclear genes. The precursor protein is synthesised in the cytoplasm and directed to the chloroplast by a targeting sequence (Keegstra and Cline, 1999). These transit peptides facilitate transport of the protein across the chloroplast envelope membranes.

29. The construct was made using the pUC19 plasmid cloning vector. The DNA fragment, containing the elements listed in Table 1, was amplified by polymerase chain reaction (PCR) and purified prior to being introduced into microalgal cells. Thus, the GMOs are expected only to contain sequences from *N. oceanica*.

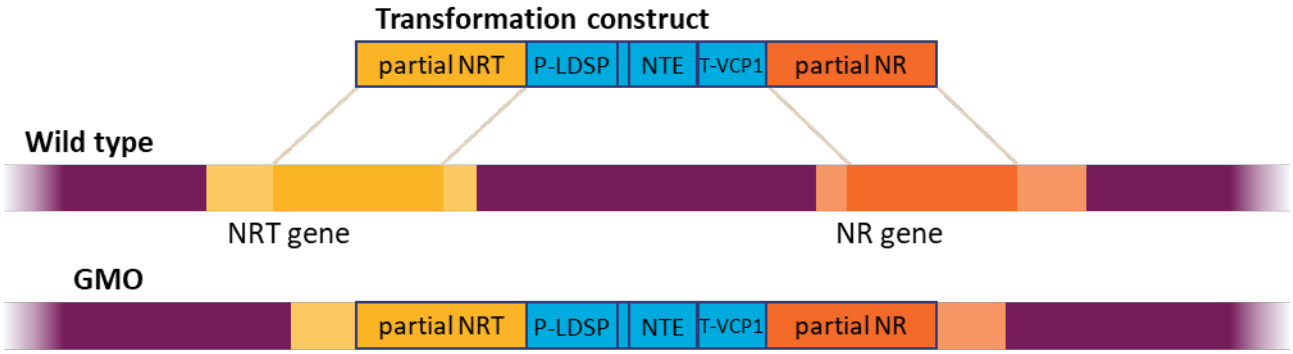

Figure 3 Integration of the transformation construct into the wild type genome via homologous recombination, to produce the GMO (not to scale). NRT gene, nitrate transporter gene; P-LDSP, promoter of lipid droplet surface protein gene; NTE, gene encoding acyl-acyl carrier protein thioesterase; T-VCP1, terminator of violaxanthin/chlorophyll a binding protein gene; NR gene, nitrate reductase gene. After Kilian et al. (2011).

Table 1 Genes and regulatory elements introduced to GM *N. oceanica* lines

Genetic element	Gene Source	Description	Function
Introduced elements			
P-LDSP	*N. oceanica* strain NIES-2145	Promoter of lipid droplet surface protein gene	Promoter sequence
VCP1SP	*N. oceanica* strain NIES-2145	Chloroplast transit sequence of violaxanthin/chlorophyll a binding protein gene	Transit peptide for import into the chloroplast
NTE	*N. oceanica* strain NIES-2145	Gene encoding acyl-acyl carrier protein thioesterase	Encoded enzyme terminates acyl elongation and increases percentage of medium-chain fatty acids
T-VCP1	*N. oceanica* strain NIES-2145	Terminator of violaxanthin/chlorophyll a binding protein gene	Terminator sequence
Partial deletions[a]			
NRT	*N. oceanica* strain NIES-2145	Nitrate transporter gene	Confers inability to use nitrate as a nitrogen source
NR	*N. oceanica* strain NIES-2145	Nitrate reductase gene	Confers inability to use nitrate as a nitrogen source

[a] Partial sequences of these genes were added to the ends of the introduced DNA fragment for homologous recombination based knock out of these genes.

4.2 The genetic modifications, and their nature and effects

4.2.1 The role of thioesterases in fatty acid biosynthesis

30. Fatty acids are made up of carbon chains of varying lengths attached to a carboxyl group. Fatty acids are important components of various cellular structures and are also used for carbon storage (Ohlrogge and Browse, 1995). *Nannochloropsis* accumulates fatty acids in the form of triacylglycerols, which are stored in lipid droplets in the cytosol (Vieler et al., 2012a).

31. Fatty acid biosynthesis in microalgae and plants occurs in the chloroplast (Ohlrogge and Browse, 1995; Radakovits et al., 2010). The elongating carbon chain is attached to an acyl carrier protein (ACP) via a sulfur atom (a thioester bond) (Ohlrogge and Browse, 1995). A series of enzyme-mediated steps increases the chain length by two carbon atoms at a time. Elongation of the carbon backbone is terminated when an acyl-ACP thioesterase removes the ACP, replacing the thioester with a hydroxyl group (Figure 4). This produces free fatty acids, which are able to leave the chloroplast (Ohlrogge and Browse, 1995).

32. Some acyl-ACP thioesterases specifically cleave off the ACP when a particular carbon chain length is reached (Radakovits et al., 2010). Overexpression of genes expressing these thioesterases can be used to alter organisms' lipid profiles.

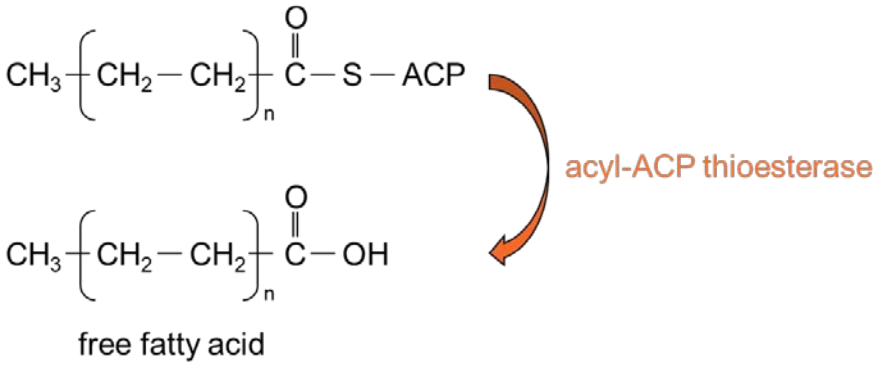

Figure 4 Acyl-acyl carrier protein thioesterase catalyses the final step in fatty acid biosynthesis.
ACP, acyl carrier protein

4.2.2 The nitrate assimilation pathway in microalgae

33. Microalgae are able to use different forms of nitrogen, such as nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+). The nitrate assimilation pathway has several steps. First, environmental nitrates are brought into the cell via a nitrate transporter (NRT). Nitrate reductase reduces nitrate to nitrite. Nitrite is further reduced to ammonium by nitrite reductase (NR), followed by enzyme-mediated assimilation into amino acids (Vieler et al., 2012b; Sanz-Luque et al., 2015).

4.2.3 The genetic modifications in the GMOs proposed for release

34. The genetic modifications are summarised in Table 1, with a description of their intended function in the GM *N. oceanica*. The introduced gene is an acyl-acyl carrier protein (ACP) thioesterase gene (NTE) for altered fatty acid production (Ozaki et al., 2015). Some amino acids in the introduced gene sequence have been changed for optimal enzyme performance.

35. The genes were introduced into a region of the *N. oceanica* genome containing nitrate assimilation genes, via homologous recombination (Kilian et al., 2011). This caused the function of the genes for a nitrate transporter (NRT) and nitrate reductase (NR) to be knocked out. The GM *N. oceanica* is thus unable to use nitrate, but can use nitrogen supplied in the form of urea or ammonium.

4.3 Toxicity/allergenicity of the proteins and fatty acids associated with the introduced genes

36. The gene sequences proposed for the release are all derived from the parent species, *N. oceanica*. The protein associated with the introduced gene is not expected to be allergenic or toxic, as there is no evidence that *N. oceanica* produces allergenic or toxic substances (see Section 3). Thioesterases are ubiquitous and are not known to be associated with toxicity or allergenicity.

37. The intended effect of the introduced gene is an increase in medium chain fatty acids, particularly C10, C12 and C14 fatty acids. *Nannochloropsis oceanica* is reported to produce fatty acids with chain lengths of C14:0, C16:n and longer (Hulatt et al., 2017). Production of trace amounts of C12 fatty acid methyl ester was reported by Mahdieh et al. (2019), when *N. oceanica* was cultivated with certain nitrogen

sources. Compared with non-GM *N. oceanica*, the GM *N. oceanica* will additionally produce capric acid (decanoic acid; C10:0) and lauric acid (dodecanoic acid; C12:0) (Ozaki et al., 2015).

38. At biological concentrations, these fatty acids are regarded as safe, e.g. these compounds are components of human breast milk (Yuhas et al., 2006) and coconut oil (Bhatnagar et al., 2009; Orsavova et al., 2015). These fatty acids are present at low concentrations in marine algae (McCauley et al., 2015).

39. According to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), the following GHS hazard statements are included by at least 50% of companies on labels for commercial pure quantities of these fatty acids (PubChem chemistry database, accessed 7 August 2019):

- Capric acid (C10:0): Causes skin irritation, Causes serious eye irritation, Harmful to aquatic life with long lasting effects.
  Toxicity data for capric acid on aquatic species is summarised in Table 2.
- Lauric acid (C12:0): Causes serious eye damage.
- Myristic acid (C14:0): *Causes serious eye irritation.*

4.4 Characterisation of the GMOs

40. The applicant has provided information from laboratory experiments comparing the GMOs with non-GM *N. oceanica*.

- Growth of both the GMO and non-GM *N. oceanica* increased with increasing salt content in F/2 media from 0–3.5%; growth was weak at 0.5% salinity and below.
- Growth of the GMO and non-GM *N. oceanica* were compared over four weeks in sterilised water from the closest freshwater creek, F/2 media, water from the Brisbane River near the proposed facility at highest possible salinity level, and seawater from Moreton Bay (Figure 5). Both the GMO and non-GM *N. oceanica* did not increase in cell density in creek water. The non-GM *N. oceanica* grew better than the GMO in F/2 media. The GMO grew better than the non-GM *N. oceanica* in river water and seawater. The applicant explained that the 2–3 fold greater growth of the GMO, compared with non-GM *N. oceanica*, in these water sources could be considered within the standard range of experimental variation, as independent outdoor experiments designed to observe logarithmic growth showed a 10–100 fold growth difference. The applicant also highlighted uncertainty around using sterilised water for growth comparison, as inter-species competition would affect both GM and non-GM *N. oceanica* growth in the natural environment.
- Growth of the GMO and non-GM *N. oceanica* were compared in Japan in brackish local river water and Wakayama seawater. The GMO did not grow better than non-GM *N. oceanica* in any of these experiments.
- Growth of the GMO and non-GM *N. oceanica* were compared in F/2 medium with either nitrate or urea as the nitrogen source. The non-GM *N. oceanica* grew well in both nitrogen sources. The GMO grew well in urea medium, but very little in nitrate medium.
- Survival of the GMO was no better than non-GM *N. oceanica* in drying experiments at temperatures of 25, 40 and 60°C for 5–60 minutes (Figure 6). These drying experiments were conducted to mimic the release of microalgae in cultivation solution onto the surrounding concrete pad and into the local environment. No microalgae survived the 60-minute drying treatments.
- Several herbicides and antibiotics were reported to be effective at controlling the microalgae, including glufosinate, glyphosate, molinate and pyraclostrobin, and hygromycin, paromomycin and zeocin, respectively. Microalgae can also be controlled using bleach (hypochlorite).
- The applicant stated that there is no evidence for increased overall lipid content in the GM *N. oceanica* lines.

Table 2 Capric acid toxicity data for some aquatic species.

Organism	Endpoint	Toxicity (mg L^{-1})	Comments[a]
Aquatic microorganisms (activated sludge)	Respiration inhibition NOEC	≥1000 (nominal)	[1] 3 h time-scale
Brine shrimp (*Artemia salina*)	LC_{50}	36	[2] 16 h
Daphnids (*Daphnia magna*)	Immobilisation, EC_{50}	16	[1] Semi-static study (48 h)
	Highest tested nominal concentration causing no mortality	10	
	NOEC	0.2	[4] Semi-static study (21 d)
Microalgae (*Anacystis nidulans*)	GI50	>100	[3] Grown for 2–3 days
Microalgae (*Chlamydomonas reinhardi*)	GI50	>100	[3] Grown for 2–3 days
Microalgae (*Chlorella vulgaris*)	GI50	>100	[3] Grown for 2–3 days
Microalgae (*Haematococcus pluvialis*)	GI50	5	[3]
Microalgae (*Navicula pelliculosa*)	GI50	50	[3] Grown for 6–8 days
Microalgae (*Pseudokirchneriella subcapitata*)	NOEC (biomass)	1.8	[4] Static study (72 h)
	E_rC_{50}	15	
Microalgae (*Scenedesmus quadricauda*)	GI50	>100	[3]
Microalgae (*S. subspicatus*)	NOE_rC	0.57	[1] Static study (72 h)
	E_bC_{50}	1.16	
	E_rC_{50}	2	
Zebrafish (*Danio rerio*; previous name *Brachydanio rerio*)	Mortality, LC_{50}	81.2	[1] Semi-static study (96 h) with octanoic acid; toxicity data was read across from octanoic acid
	LC_0	55	
	NOEC	23.6	
	LC_{50}	9.8	[4] 28 d; flow-through
	NOE_rC	6.4	
	Mortality, NOEC	2	

EC_{50}, median effective concentration; E_bC_{50}, median effective concentration (biomass); E_rC_{50}, median effective concentration (growth rate); GI50, growth inhibition by approximately 50%; LC_0, lethal concentration 0%; LC_{50}, median lethal concentration; NOEC, no observed effect concentration; NOE_rC, no observed effect concentration (growth rate).

[a] References: [1] ECHA (2013), [2] Curtis et al. (1974), [3] Proctor (1957), [4] European Chemicals Agency website (accessed 11 December 2019).

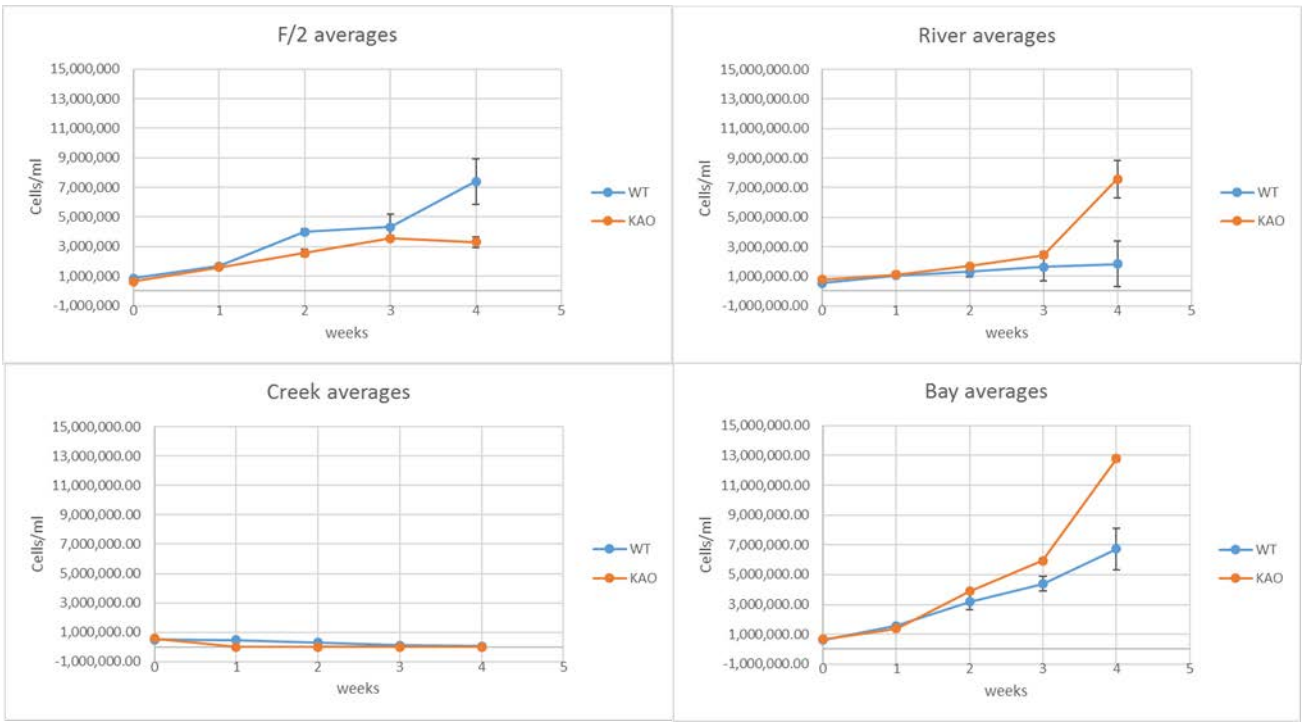

Figure 5 Growth of wild type (WT) *N. oceanica* and GM *N. oceanica* (KAO) in sterilised water from sources near the proposed trial site.
F/2, microalgal culture medium; River, water taken from the Brisbane River near the pilot plant site at high king tide; Creek, water from the closest freshwater creek; Bay, seawater taken from Moreton Bay, approximately 20kms from the site**.** Results are from two independent experiments, each with triplicate samples.

		Drying time (min)					
		0	5	10	15	30	60
25 °C	WT	●	●	●	●	△	×
	GM	●	●	●	●	×	×
40 °C	WT	●	●	×	×	×	×
	GM	●	●	×	×	×	×
60 °C	WT	●	×	×	×	×	×
	GM	●	×	×	×	×	×

● Survived drying treatment
△ Partially survived
× Did not survive

Figure 6 Ability of wild type (WT) and GM *N. oceanica* (GM) to survive drying at 25°C, 40°C and 60°C for periods of time ranging from 0–60 minutes.

41. The applicant has stated that the GM *N. oceanica* have been modified to produce increased amounts of medium-chain fatty acids (C10, C12 and C14), but has not supplied data to show how the fatty acid profile of the GM *N. oceanica* is different from non-GM *N. oceanica*. The applicant has confirmed that they have not observed greater concentrations of capric acid in any GM *N. oceanica* proposed for release than in any GM lines used in their patent. An example of the change in fatty acid profiles in *N. oceanica* genetically modified with NTE is found in a patent by Ozaki et al. (2015). As shown in Figure 7, the GM *N. oceanica* lines used in the patent produce an increased proportion of lauric acid (12:0) and myristic acid (C14:0), with a reduction in the proportion of long-chain palmitic acid (C16:0).

42. The applicant has reported that no adverse health effects have been observed in staff working with GM *N. oceanica.*

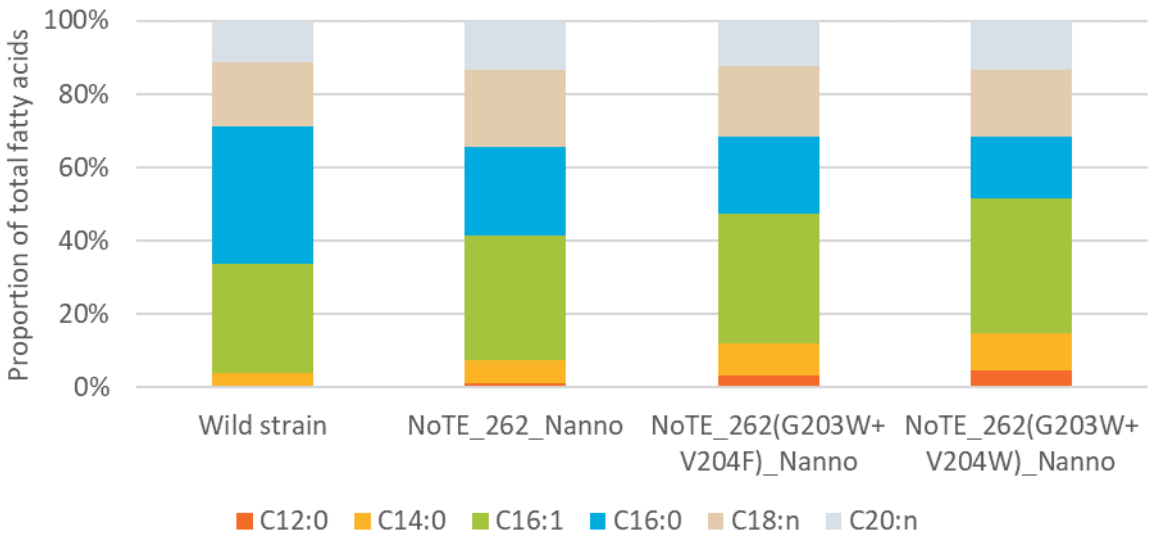

Figure 7 Fatty acid composition of wild strain *N. oceanica* compared with GM *N. oceanica* cell lines with an inserted NTE gene, from Table 8, Ozaki et al. (2015).
NoTE_262_Nanno, *N. oceanica* strain NIES2145 with NTE gene; (G203W+V204F) and (G203W+V204W) are NTE gene variants with codons replaced at the specified position, e.g. in G203W the codon encoding glycine at position 203 was replaced with a codon encoding tryptophan.

Section 5 The receiving environment

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

44. Information relevant to the cultivation and distribution of *N. oceanica* in Australia, including key biotic and abiotic interactions in the *N. oceanica* growing environment, is presented in the *N. oceanica* biology document (OGTR, 2019).

5.1 Relevant abiotic factors

45. The proposed release would be carried out at one site, at UQ's Pinjarra Hills campus (Centre for Solar Biotechnology pilot plant), Brisbane City, Qld. The GM *N. oceanica* would be cultivated in up to six culture vessels, each with up to 600 litre cultivation volume.

46. *Nannochloropsis oceanica* grows in saline water. According to the Business Queensland watercourse identification map (accessed 9 August 2019), the pilot plant facility is 172 m from the nearest freshwater lake, 97 m from the nearest watercourse/drainage feature, and 799 m from the Brisbane River. The nearest property connected to sewerage is over 500 m from the site (Queensland Urban Utilities, 2017).

47. The proposed release site at Pinjarra Hills is approximately 46 km upstream from the mouth of the Brisbane River (Yu et al., 2014). At this location, Brisbane River water is brackish. The tidal limit of the Brisbane River extends approximately 34 km further upstream from Pinjarra Hills.

48. According to the Business Queensland FloodCheck online map (accessed 16 August 2019), the facility is 40–50 m above sea level and outside historic flood lines, including the *Brisbane Ipswich Floods 1974*, *Brisbane Floods 1893* and *2010 to 2011 Interim Flood Lines*.

49. Weather and climate data for Archerfield Airport, 10 km southeast of Pinjarra Hills, was obtained from the Bureau of Meteorology (Climate Data Online, accessed 9 August 2019). Highest daily rainfall between 1929 and 2019 was recorded at 343.7 mm on 6 February 1931. The maximum wind gust between 1939 and 2019 was 143 km h^{-1} on 15 December 1946. For the year July 2018 – June 2019, the maximum

wind gust was 83 km h^{-1}. Maximum wind gusts were most often recorded coming from a north-north-easterly direction (Figure 8). The Brisbane River runs along the southern and eastern sides of the Pinjarra campus (Figure 9). The closest marine waters are in Moreton Bay, which is in a north-easterly direction from the proposed site.

50. The region is not directly impacted by tropical cyclones, but can receive heavy rainfall from ex-tropical cyclones.

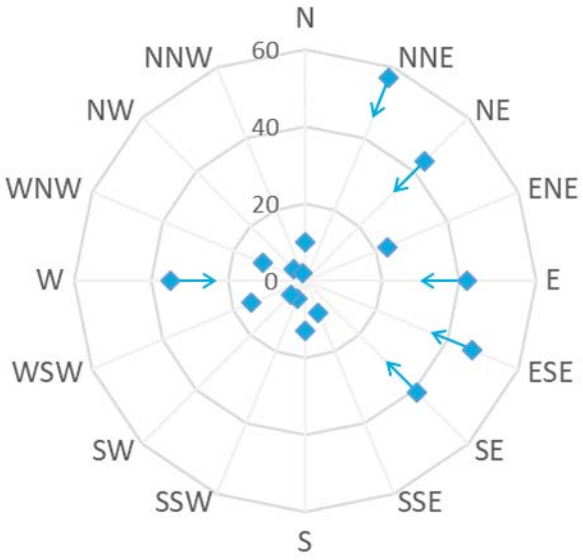

Figure 8 Number of days that maximum wind gusts came from a particular direction at Archerfield Airport, Qld, for the year July 2018 – June 2019.

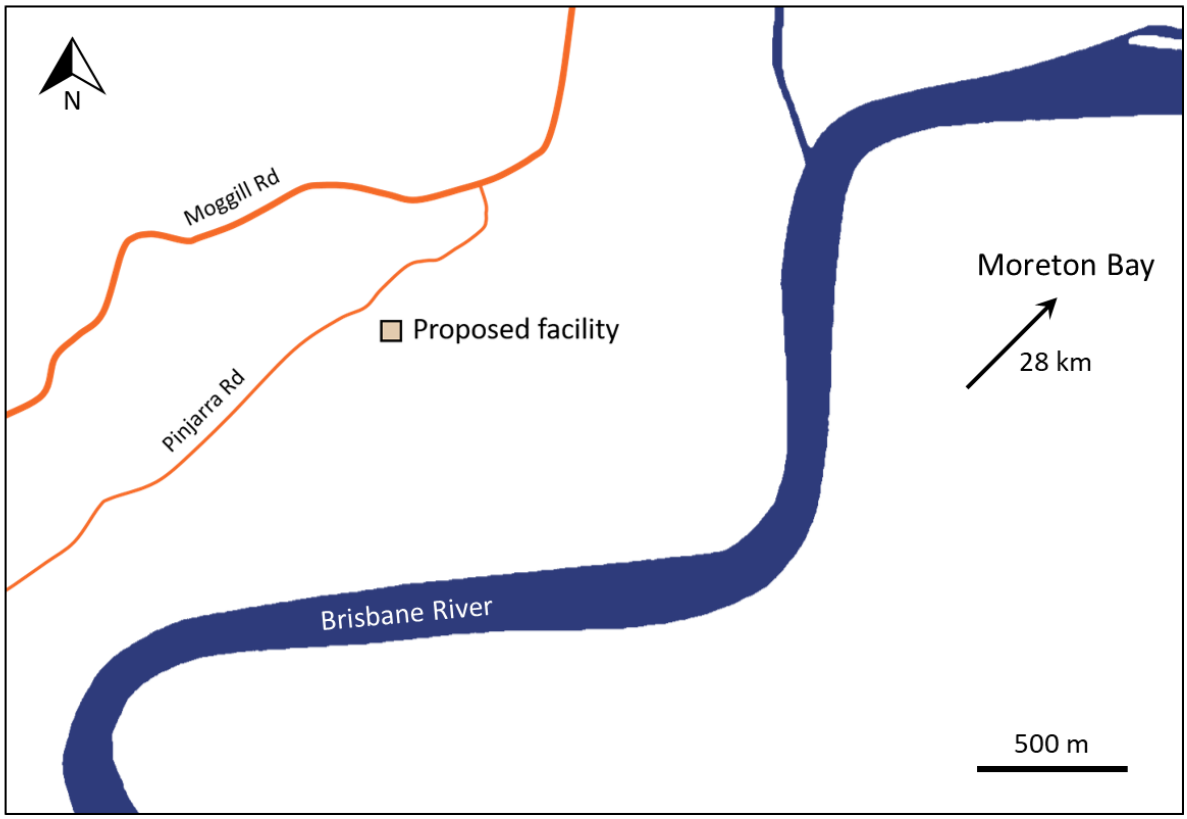

Figure 9 Location of the proposed facility at UQ's Pinjarra Hills campus in relation to the Brisbane River and Moreton Bay.

Nitrogen sources in nearby waterways

51. Nitrogen enters river systems by various means, including deposition of atmospheric nitrogen, surface runoff of fertilisers and via groundwater (Xia et al., 2018). Nitrates are the predominant form of inorganic nitrogen in river systems. Nitrates are transformed by microorganisms via nitrification and denitrification (Figure 10). Nitrification is mediated by aerobic bacteria , while denitrification generally requires low oxygen conditions (Xia et al., 2018). This means that the two processes tend to occur at different depths in the river, i.e. in overlying water and sediments, respectively. The concentration of different forms of nitrogen also varies with depth (Seitzinger, 1988).

52. Water analysis data provided by the applicant showed that nitrogen sources in Brisbane River water, from near the pilot plant site, were 850 ppb nitrate[5], with no detectable nitrite or ammonium. Water in Moreton Bay contained 70 ppb ammonium, and no detectable nitrite or NO_x (nitrate + nitrite).

53. Water analysis data from the mouth of the Brisbane River in 2014 showed that the most abundant form of nitrogen was always nitrate (BMT WBM, 2015). Nitrate was measured in the Brisbane River estuary at concentrations of 22–140 µg L^{-1} in February and 74–150 µg L^{-1} in August; nitrite was below the detection limit in February and present at 9–21 µg L^{-1} in August; ammonia was below the detection limit in February and present at 10 µg L^{-1} in one sample in August.

54. Water analysis data from Moreton Bay open coastal waters in 2014 also shows that the most abundant form of nitrogen was nitrate (BMT WBM, 2015). Nitrate was measured at concentrations of 18–33 µg L^{-1} in February and up to 59 µg L^{-1} in August; nitrite was below the detection limit in February and present at up to 14 µg L^{-1} in August; ammonia was below the detection limit in February and August.

55. Although many water quality surveys only measure inorganic sources of nitrogen, a significant proportion of nitrogen can be available in organic forms. Water analysis data from Moreton Bay in 1997-98 showed that up to 55% of dissolved nitrogen was in the form of urea in the eastern bay region, with nitrate sometimes only contributing 6% of available nitrogen (Glibert et al., 2006). In an inland tributary of the Brisbane River, however, dissolved nitrogen was almost entirely present in the form of nitrate, with only trace levels of ammonium and urea present.

56. The GM *N. oceanica* are able to grow in water from the Brisbane River and from Moreton Bay (see Section 4.4). This indicates that non-nitrate sources of nitrogen are available in these waters.

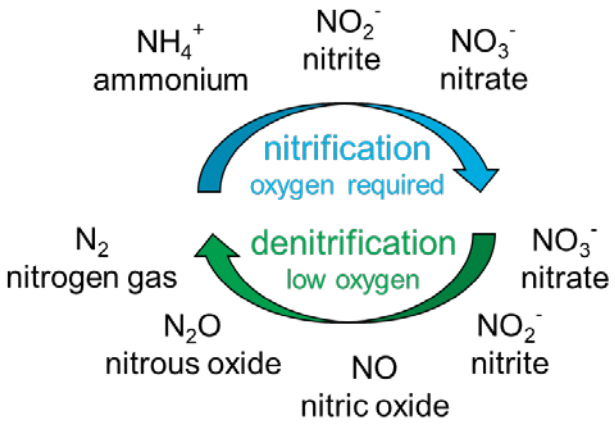

Figure 10 The chemical pathways leading to nitrification and denitrification of nitrates in waterways.

[5] The data provided showed 850 ppb as NO_x-N (nitrate + nitrite); however, the nitrite concentration was 0 ppb. 850 ppb is equivalent to 850 µg L^{-1}.

5.2 Relevant biotic factors

57. *Nannochloropsis oceanica* does not reproduce sexually.

58. *Nannochloropsis* sp. has been isolated from the Brisbane River, approximately 11 km from the proposed facility and *N. oceanica* has been isolated from Deception Bay, Qld, indicating that these organisms have an established role in the ecology of southern Qld marine waters.

59. The applicant has supplied information about animal activity at the proposed facility, including images from a closed circuit television camera. Birds visit the facility during the day. Insect activity occurs predominantly at night. Mice were detected on the concrete pad during the night.

60. Under non-sterile culture conditions, microalgae are closely associated with bacteria, which may have beneficial or inhibitory effects on the culture. Large-scale open cultures are more prone to contamination with other microalgae and predatory microorganisms than closed systems (OGTR, 2019).

5.3 Relevant cultural practices

61. Large-scale microalgal production facilities tend to be located in coastal regions of Australia (OGTR, 2019). Microalgal production in Australia occurs at a smaller scale than other industries, such as crop or livestock production. Different species of microalgae are produced, requiring different culture conditions. The CSIRO's Australian National Algae Culture Collection (ANACC, accessed 19 August 2019) supplies microalgal starter cultures. Methods for microalgae culture are available via the ANACC website or from other organisations, e.g. FAO (1996).

62. Details of the cultural practices proposed by the applicant are as follows:

- Inoculum of GM *N. oceanica* would be cultured in 20-litre plastic culture bags in a PC2 certified facility adjacent to the pilot plant. Culture bags would be transferred to the pilot plant facility and hung for a full day and night, in order for the microalgae to acclimatise to outdoor conditions, prior to inoculation of the culture vessels.
- Culture vessels containing appropriate growing media would be inoculated with GM *N. oceanica* from a sealed container via a plastic tube fitted with a tap. Cultures would typically be grown for two weeks prior to harvest. Trained staff are generally present at the facility five days per week.
- Culture vessels used for cultivating the GM microalgae would be covered with a clear plastic lid, which are only removed in an emergency. Piping, cooling loops, gassing units, and sampling and sensor ports are fitted to the closed culture vessel with valves, so that lids do not need to be removed during a run. Probes (e.g. pH probes) and cooling loops inserted into the culture vessels would be inserted via sealable ports, and can be sterilised after removal from the culture vessels.
- Samples of microalgal culture (1–500 mL) would be collected from the culture vessels daily.
- The microalgae need carbon dioxide (CO_2) to grow; this would be supplied in the form of air, CO_2-enriched air or CO_2. Air would be released from the culture vessels during this process; supply and outlet lines could be filtered using a cell free filter to reduce the potential for aerosol release.
- At harvest, controlled piping and pump systems would be used to transfer cultures to a centrifuge in a shed at the facility. Maintenance is performed prior to every use of the pumps and pipes to prevent leaks occurring. Containers would be placed under pipe connection points to collect spills, which would be treated with hypochlorite prior to disposal. The culture vessels and hoses are located on a concrete pad that is painted white, and any leaks of microalgal solution are visible.
- Microalgal cells would be harvested using a continuous disk stack centrifuge. Waste water would pass through a sterile filtration system prior to collection in a waste water tank.
- Following harvest, all equipment and culture vessels would be sterilised prior to reuse. Culture vessels would be treated with hypochlorite while lids remain closed. The hypochlorite solution would then be flushed out of the culture vessel through the hypochlorite delivery pipe to a cell free filter unit and collected in a waste water tank. The water in the waste water tank would be monitored for GMO growth before water is released from the pad.
- Harvested biomass would be transported in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* to PC2 facilities for analysis.

5.4 Presence of related microalgae in the receiving environment

63. In addition to the six culture vessels that would be used for the cultivation of the GM *N. oceanica*, a further two open raceway ponds (each with 2500 litre cultivation volume) and twelve closed bioreactors are located at the pilot plant facility. The applicant proposes to put lids on the raceway ponds should they culture non-GM microalgae while GM *N. oceanica* is grown in the other culture vessels. They also propose to treat all microalgae grown at the site like the GMO while GM *N. oceanica* is cultivated.

64. Open culture vessels for microalgal cultivation are also located on the Pinjarra Hills campus at a distance of approximately 1.3 km from the proposed facility. The cultivation media used in these culture vessels and bioreactors would be suitable for *N. oceanica* growth. *Nannochloropsis* sp. could be cultivated in these vessels at the same time that the GM *N. oceanica* is grown.

65. *Nannochloropsis oceanica* has been isolated from Deception Bay, Qld, and is expected to be present in the coastal waters approximately 28 km from the proposed facility. *Nannochloropsis* sp. was isolated, using seawater growth medium, from the Brisbane River approximately 11 km downstream from the proposed trial site (see Chapter 1, Section 3).

66. *Nannochloropsis oceanica* does not grow in freshwater or terrestrial environments.

5.5 Presence of similar genes and encoded proteins in the environment

67. The introduced gene and regulatory sequences were derived from the parent species, *N. oceanica*, which is widespread in the marine environment (see Table 1, section 4.1).

68. As discussed in Section 4.2, thioesterases are an integral part of fatty acid biosynthesis and are widespread in microalgae and plants. Therefore, it is expected that humans, animals and microorganisms routinely encounter the introduced gene for thioesterase, or homologues of this gene and its expressed protein (or proteins with a similar function), through contact with microalgae, plants, and food derived from plants.

69. The regulatory sequences that control expression of the genes inserted in the GM *N. oceanica* are derived from the parent organism, as described in Section 4.1.

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

70. There have been no approvals for trials or commercial release of GM *N. oceanica* in Australia.

6.2 International approvals

71. There have been no approvals for release of GM *N. oceanica* in any country.

72. A field trial of GM microalgae (*Scenedesmus dimorphus*) in open ponds was approved by the United States Environmental Protection Agency in September 2013 (US EPA TERAs R-13-0003 to R-13-0007; accessed 16 July 2019). The species is a freshwater microalga in the kingdom Plantae. The trial included experiments to test the ability of the microalgae to disperse aerially at the field site (Szyjka et al., 2017). There were no reports of harm from this trial.

Chapter 2 Risk assessment

Section 1 Introduction

73. 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 11). 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.

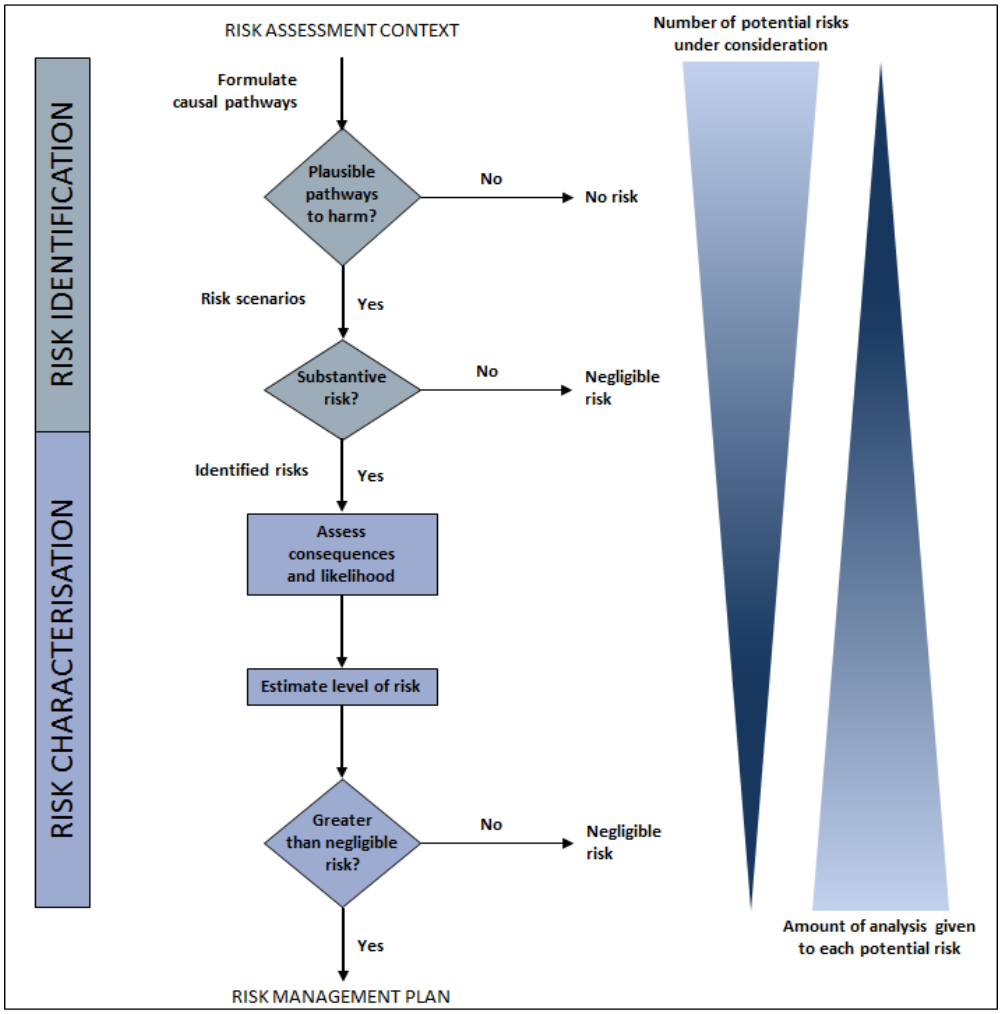

Figure 11 The risk assessment process

74. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, reported international experience and consultation (OGTR, 2013). Risk scenarios examined in RARMPs prepared for licence applications for the same or similar GMOs, are also considered.

75. Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating plausible causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are risk scenarios. These risk scenarios are screened to identify those that are considered to have a reasonable chance of causing harm in the short or long term. Pathways that do not lead to harm, or those that could not plausibly occur, do not advance in the risk assessment process (Figure 11), i.e. the risk is considered to be no greater than negligible.

76. Risks identified as being potentially greater than negligible are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). Risk

evaluation then combines the Consequence and Likelihood assessments to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 Risk Identification

77. Postulated risk scenarios are comprised of three components (Figure 12):

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.

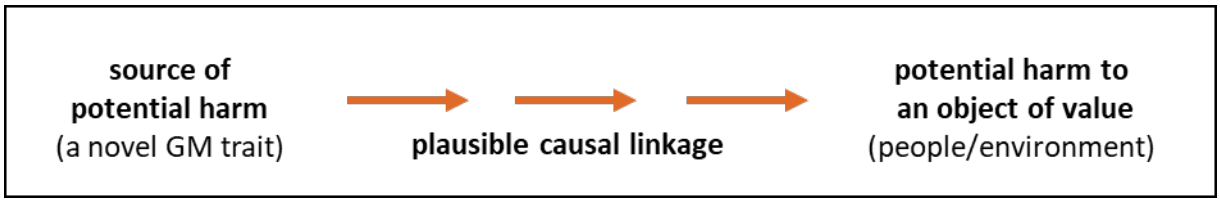

Figure 12 Risk scenario

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

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

2.1 Risk source

79. 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.

80. As discussed in Chapter 1, the GM *N. oceanica* are modified by the introduction of the NTE gene, derived from *N. oceanica*, and by the partial deletion of the NRT and NR genes. The intended effect of insertion of the NTE gene is to increase production of medium chain fatty acids. The intended effect of the partial gene deletions is to prevent the GM *N. oceanica* from being able to use nitrates. These modified genes are considered further as potential sources of risk.

81. The introduced gene is controlled by introduced regulatory sequences. These are derived from *N. oceanica*. Regulatory sequences are naturally present in all microalgae and the introduced sequences are expected to operate in similar ways to endogenous sequences. These sequences are DNA that is not expressed as a protein, so exposure is to the DNA only and dietary DNA has no toxicity (Society of Toxicology, 2003). Hence, the introduced regulatory sequences will not be further considered as sources of potential harm for this application.

Unintended effects of the process of genetic modification

82. Genetic modifications have the potential to cause unintended effects in several ways. These include insertional effects, where the introduced genetic elements are randomly integrated into the host's genome. These effects, such as interruptions, deletions, duplications or rearrangements of the genome, can lead to altered expression of endogenous genes (Schnell et al., 2015). However, in the GMOs proposed for release, the genetic elements that have been inserted and removed are at a known location of the GM *N. oceanica* genome and there are no known disruptions to other endogenous genes. The potential for the processes of genetic modification to result in unintended effects will not be considered further.

2.2 Causal pathway

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

- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)
- potential effects of the introduced gene(s) and gene product(s) on the properties of the organism
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment
- the environment at the site(s) of release
- 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. water quality)
- tolerance to biotic stressors (e.g. pests, pathogens and weeds)
- tolerance to cultivation management practices
- potential for gene transfer to sexually compatible organisms
- potential for gene transfer by horizontal gene transfer
- potential for unauthorised activities.

84. Although all of these factors were considered, some are not included in the risk scenarios below as a plausible pathway to harm could not be identified.

Vertical gene transfer

85. As discussed in Chapter 1, Section 3, *N. oceanica* is unable to reproduce sexually. Therefore, the potential of gene transfer via sexual reproduction as part of a pathway to harm will not be assessed further.

Horizontal gene transfer

86. The potential for horizontal gene transfer (HGT) from GMOs to species that are not sexually compatible, and any possible adverse outcomes, have been reviewed in the literature (Keese, 2008) and assessed in many previous RARMPs, e.g. in the RARMP for DIR 108, a GM canola. The frequency of HGT is dependent on several factors, with single celled microalgae having a higher relative HGT frequency than multicellular eukaryotes, such as plants (Keese, 2008; Beacham et al., 2017). The environment and relationships of microorganisms are also factors. These include symbiotic relationships such as those between microalgae and bacteria, and between microalgae and their viruses (Beacham et al., 2017).

87. Eukaryotic microalgae are less capable of HGT than cyanobacteria and were thus considered by Henley et al. (2013) as more suitable candidates for genetic modification. However, HGT has been documented in both directions between a eukaryotic microalga and its DNA virus (Monier et al., 2009). Horizontal gene transfer of a gene involved in photosynthesis has been shown to take place over evolutionary time frames from *Vaucheria litorea* to the sea slug *Elysia chlorotica*, which consumes the algae as part of its food source (Rumpho et al., 2008). Like *Nannochloropsis*, *V. litorea* belongs to the Ochrophyta phylum. No evidence for HGT specifically from *Nannochloropsis* to other organisms has been identified (OGTR, 2019).

88. In this application, the genetic elements introduced in *N. oceanica* strain CS-264 all originate from the *N. oceanica* strain NIES-2145 and as such, are present, widespread and available in the environment for transfer via demonstrated natural mechanisms. In addition, microalgal waste will not be released into the environment, so HGT to soil microorganisms will not be a concern. Therefore, the potential of HGT as part of a pathway to harm will not be further considered for this application.

Deletions conferring inability to use nitrates

89. The NRT and NR genes have been partially deleted from the *N. oceanica* genome, conferring an inability for the GM microalgae to use nitrates. The intended effect of this deletion is to reduce the fitness of the GM microalgae, compared with non-GM *N. oceanica.*

90. A potential effect of the deletion is selective use of environmental nitrogen, leading to a change in the ratio of nitrogen compounds available for organisms in the environment, and a subsequent impact on the quality of ecosystems.

91. Another potential effect is an increase in total fatty acid content, as a result of nitrogen limitation. Although the applicant has stated that there is no evidence for increased total lipid content, lipid content of *N. oceanica* can be manipulated by changing nutrient conditions (Hulatt et al., 2017).

92. Available data indicates that nitrate is the predominant form of nitrogen in Brisbane River water, with lower levels in Moreton Bay water (see Chapter 1, Section 5.1). The concentration of different nitrogen sources fluctuates greatly, both spatially and temporally (Glibert et al., 2006; BMT WBM, 2015). Thus, any adverse effect of the selective use of certain nitrogen sources by the GM *N. oceanica* would occur within the context of natural fluctuations and will not be considered further.

Unauthorised activities

93. The potential for unauthorised activities to lead to an adverse outcome has been considered in many previous RARMPs, most recently in the RARMP for DIR 117. In previous assessments of unauthorised activities, no substantive risk was identified. The Act provides substantial penalties for unauthorised dealings with GMOs or noncompliance with licence conditions, and also requires the Regulator to have regard to the suitability of an applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, risks from unauthorised activities will not be considered further.

2.3 Potential harm

94. Potential harms from GM microalgae are based on those used to assess the weed risk from aquatic and land-based plants (Virtue, 2008; Keese et al., 2014), and those specifically proposed for microalgae (Henley et al., 2013), including:

- harm to the health of people or other desirable organisms, including toxicity/allergenicity
- reduced biodiversity through harm to other organisms or ecosystems
- reduced ecosystem services (e.g. degradation of drinking water sources or recreational waters, negative effects on fisheries)
- reduced quality of the biotic environment (e.g. harmful algal blooms, providing food for pests or pathogens) or abiotic environment (e.g. negative effects on nutrient levels).

95. Formulation of risk scenarios for GM microalgae based on risks posed by GM crop plants is considered a valid approach, as risk evaluation for GM crop plants is well established (Henley et al., 2013). It should be noted that 'microalgae' are a far more diverse group of organisms than crop plants, with species of microalgae occurring in four kingdoms of living organisms (OGTR, 2019).

96. Judgements of what is considered harm depend on the environment where the GM *N. oceanica* may be present. A species of microalgae may pose a different risk to different environments, such as in culture vessels on a microalgae farm; a marine environment currently used for commercial fishing, mining or oyster farming; or a marine park.

2.4 Postulated risk scenarios

97. Two risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in the table below and examined in detail in Sections 2.4.1 – 2.4.2.

98. In the context of the activities proposed by the applicant and considering both the short and long term, neither of the two risk scenarios gave rise to any substantive risks.

Table 3 Summary of risk scenarios from the proposed dealings with the GM microalgae

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	Introduced gene conferring increased production of certain fatty acids	Cultivation of GM *N. oceanica* expressing the introduced gene ⬇ Increased or novel production of certain fatty acids in GM *N. oceanica* ⬇ Exposure of people or other desirable organisms to the GM *N. oceanica* at the pilot plant facility	• Increased toxicity or allergenicity for humans • Increased toxicity to other desirable organisms	No	• The introduced gene was originally derived from *N. oceanica* which is not known to be toxic or allergenic. • The introduced protein belongs to a protein family which is ubiquitously present in plants and microalgae, as well as the food derived from them, and which has no known toxicity or allergenicity. • Medium chain fatty acids occur naturally in the environment, and are not known to be toxic at the expected concentrations. • The limits and controls of the proposed trial would minimise exposure. • The GM *N. oceanica* from this trial would not be used in food or feed.
2	Introduced gene conferring increased production of certain fatty acids	Cultivation of *N. oceanica* expressing the introduced gene ⬇ Presence of GM *N. oceanica* outside the trial limits ⬇ Establishment and persistence of GM *N. oceanica* in a suitable aquatic environment	• Increased toxicity or allergenicity for humans • Increased toxicity to other desirable organisms • Reduced quality of the biotic environment • Reduced establishment of desirable organisms	No	• The proposed limits and controls would minimise the likelihood of dispersal of the GM *N. oceanica*. • The altered fatty acid profile is not expected to increase the toxicity of the GM *N. oceanica* compared with non-GM *N. oceanica.*There is no expectation the introduced gene confers an advantage to the GM *N. oceanica*. • *Nannochloropsis oceanica* is not known to form harmful algal blooms or cause other harmful effects, and there is no reason to expect the GM *N. oceanica* to behave differently.

2.4.1 Risk scenario 1

Risk Source	Introduced gene conferring increased production of certain fatty acids
Causal Pathway	⬇ Cultivation of *N. oceanica* expressing the introduced gene ⬇ Increased or novel production of certain fatty acids in GM *N. oceanica* ⬇ Exposure of humans or other desirable organisms to the GM *N. oceanica* at the pilot plant facility ⬇
Potential Harm	Increased toxicity or allergenicity for humans OR Increased toxicity to other desirable organisms

Risk source

99. The source of potential harm for this postulated risk scenario is the introduced gene for increased production of certain fatty acids in the GM *N. oceanica*.

Causal pathway

100. The GM *N. oceanica* is cultivated at the pilot plant and the thioesterase gene is expressed, resulting in the novel or increased production of the fatty acids capric, lauric and myristic acids.

101. People may be exposed to GM *N. oceanica*, the expressed protein or medium chain fatty acids either by direct contact with the GM *N. oceanica* culture solution or by inhalation of aerosolised GM *N. oceanica*. This would most likely occur when people are working with GM *N. oceanica*, e.g. while monitoring growing conditions, during harvest and during post-harvest cleaning. Although ingestion of GM *N. oceanica* would be possible, this is highly unlikely to occur in the proposed trial because microalgal culture is not a recognised food for people, and the culture medium is considered not to induce people to taste it as it is saline.

102. Other desirable organisms entering the pilot plant facility, such as mammals, e.g. rodents, birds or invertebrates, e.g. snails, may be exposed via contact with, inhalation or ingestion of the GM *N. oceanica* culture solution. Similar to people, land-based animals would not be inclined to ingest the saline culture medium. The culture would be maintained in a manner to avoid the introduction of any contaminating organisms. Thus, any aquatic organisms other than the GMOs would be considered undesirable in the culture vessels; however, due to the proposed controls, aquatic organisms are also unlikely to be exposed to the GMOs.

103. As mentioned in Chapter 1, Section 3, microalgae are readily aerosolised, e.g. by bubbles bursting in foams or in aerated culture vessels such as those that would be used in the proposed trial. This could lead to inhalation of aerosolised GM *N. oceanica*. Leaks during culturing, harvest or post-harvest cleaning may lead to dermal contact of people or other desirable animals with the GM *N. oceanica*.

104. The GM *N. oceanica* is proposed to be cultivated over several periods of time up to a total of 12 months. The potential for exposure is limited to the periods during which the GMOs are present at the pilot plant facility.

105. The GM *N. oceanica* would be grown in batches for approximately two weeks per batch. Growing of the GM *N. oceanica* would occur in six culture vessels, each with a volume of up to 600 litres. Although the culture vessels are located outside on a concrete pad, each vessel would be covered with a securely fitted lid while the GM *N. oceanica* is grown. Other measures to avoid dispersal via aerosols, through leaks of culture medium or in waste would also be in place during cultivation, harvest, and post-harvest cleaning (see Chapter 1, Section 5.3).

106. Permission to access the site would only be given to authorised and appropriately trained people.

107. Transport and storage of the GM microalgae would be conducted according to the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*, thus limiting exposure of people during transport and storage of the GMOs.

108. No material from this trial would be used for human food or animal feed. These proposed limits and controls would minimise the exposure of people and animals to the GM *N. oceanica*.

Potential harm

109. Toxicity is the adverse effect of exposure to a substance (Klaassen and Watkins, 2010). The effect of a toxic agent depends on the dose, duration of exposure and exposure route, e.g. inhalation, ingestion or via the skin. Responses may be either immediate or delayed. Allergic reactions are a type of adverse effect, resulting from sensitisation to a chemical, followed by an allergic response upon subsequent exposure. Allergenicity is the potential for a chemical to be recognised by the body as a foreign substance and to elicit a (disproportionate) immunological reaction.

110. Potentially, people exposed to the GM *N. oceanica*, including the introduced thioesterase gene or protein, or to the fatty acids produced by the thioesterase may show increased toxic reactions or increased allergenicity when compared with exposure to non-GM *N. oceanica*. Similarly, exposure to the GM *N. oceanica*, including the introduced gene, protein or fatty acids, may lead to increased toxicity to other desirable organisms.

111. No toxicity studies have been undertaken by the applicant regarding the GM *N. oceanica.* Therefore, this is an area of uncertainty for this risk assessment. Data from other sources is presented below.

Potential for toxicity or allergenicity to people and toxicity to other desirable organisms from the introduced thioesterase gene and protein

112. The source organism of the introduced gene is *N. oceanica*, which is not known to be toxic or allergenic to people, or toxic to other desirable organisms (OGTR, 2019). While some amino acids in the introduced gene have been changed to improve enzyme performance, the expressed protein still functions as a thioesterase.

113. There is no reasonable expectation that the introduced gene or protein may result in an increased level of harm when compared with non-GM *N. oceanica.* There is also no reasonable expectation that the introduced gene or protein would affect pathways producing toxins or allergens in *N. oceanica*, or lead to the production of novel toxins or allergens. While no toxicity or allergenicity studies have been performed on the GM *N. oceanica* or the introduced protein, the applicant has stated that no adverse health effects have been observed in staff working with GM *N. oceanica.*

114. In addition, although widespread, thioesterase proteins are not known to be toxic or allergenic, e.g. thioesterase from *Umbellularia californica* is not identified as allergenic by computational allergy prediction (Verma et al., 2011). Fatty acid biosynthesis in chloroplasts involving thioesterase activity occurs widely in microalgae and plants (Ohlrogge and Browse, 1995; Radakovits et al., 2010). As such, humans and other beneficial organisms routinely encounter the introduced gene or homologues of the gene and its product through contact with microalgae or plants, and food derived from them without known ill effects.

115. Although allergic reactions have been reported to some freshwater microalgae, with sensitisation likely occurring via inhalation of airborne cells (OGTR, 2019, and references therein), there are no reports of allergic reactions to non-GM *N. oceanica.*

Fatty acid composition in the GM Nannochloropsis oceanica

116. The introduced thioesterase is expected to alter the ratio of fatty acids in GM *N. oceanica* cells. The applicant has stated that the concentration of capric (C10:0), lauric (C12:0) and myristic (C14:0) acids is expected to increase, but that the total lipid content would remain the same as in non-GM *N. oceanica*. The increase in medium chain fatty acids might be offset by a decrease in palmitic acid (C16:0) concentration (Figure 7; Ozaki et al., 2015).

117. Non-GM *N. oceanica* already produce myristic acid (Hulatt et al., 2017); however, the production of capric acid and lauric acid may be novel to the GM *N. oceanica*.

118. Capric acid and lauric acid concentrations in GM *N. oceanica* cells are expected to be relatively low, at approximately 2% of dry weight each. Myristic acid is expected to reach approximately 4% of dry weight. These figures are based on information on fatty acid composition of wild type *N. oceanica* and on information from a patent on which this work is based, as discussed in the following paragraph. The applicant has confirmed that they have not measured greater concentrations of capric, lauric or myristic acids in the GM *N. oceanica*.

119. Total fatty acid (TFA) composition of *N. oceanica* is in the order of 100–400 mg g^{-1} dry weight (Hulatt et al., 2017; OGTR, 2019). Lauric acid is expected to increase, up to approximately 5% of TFA, in the GM *N. oceanica* (Ozaki et al., 2015). Based on expression studies in *Escherichia coli*, any production of capric acid is expected to be in the range of lauric acid or lower. Thus, the concentration of each acid could reach 20 mg g^{-1} or 2% of dry weight. Myristic acid is expected to increase from approximately 4% of TFA, up to approximately 10%.

120. It is not expected that growth of the GM *N. oceanica* in outdoor culture vessels would result in greater medium chain fatty acid concentrations than measured in the laboratory. In similar work by Szyjka et al. (2017), a thioesterase gene for enhanced myristic (C14:0) acid production was introduced into *Scenedesmus dimorphus* microalgae. The authors reported that the effect of the modification, i.e. increased myristic acid production, was greater when microalgae were grown under laboratory conditions than in open ponds.

Properties of medium chain fatty acids

121. There is a trend towards increasing toxicity and irritating properties as fatty acid chain lengths decrease (ECHA, 2013).

122. In its pure form, capric acid causes serious eye irritation, skin irritation and is harmful to aquatic life with long lasting effects (ECHA, 2013). Undiluted capric acid is a skin irritant when tested on rabbit skin, human skin and *in vitro* (Jirova et al., 2008). Prolonged skin exposure to 8.6% capric acid caused skin reddening in humans after 2–8 days; while 17.2% capric acid did not cause irritant reactions after contact exposure for 24 hours (NICNAS, 2001, and references therein).

123. Capric acid is safe for use in foods at lower concentrations. When rats were fed for 150 days with rice containing 10% added capric acid, no damage to forestomachs or glandular stomachs was observed (Mori, 1953). Capric acid has been used as a flavouring ingredient at concentrations up to 9.9 parts per million[6] in foods and beverages, at which concentration it is generally recognised as safe (Hall and Oser, 1965). The flavour of capric acid is described as sour, citrus, fat, rancid, fatty, unpleasant, dust and grass (FlavorDB, accessed 27 August 2019). Capric acid is not known to be allergenic.

124. According to the European Chemicals Agency (accessed 27 September 2019), pure lauric acid causes serious eye damage. However, lauric acid has been used in foods and beverages as a flavouring ingredient at concentrations up to 39 parts per million and is generally recognised as safe (Hall and Oser, 1965). The flavour of lauric acid is described as coconut, mild, fatty, metal and bay oil (FlavorDB, accessed 27 August 2019). Lauric acid is not known to be allergenic.

125. According to the European Chemicals Agency (accessed 27 September 2019), pure myristic acid causes serious eye irritation and causes skin irritation. At concentrations up to 13%, myristic and lauric acids are not primary or cumulative irritants, nor sensitisers (Becker et al., 2010). Myristic acid has been used as a flavouring ingredient at concentrations up to 10 parts per million in foods and beverages, at which concentration it is generally recognised as safe (Hall and Oser, 1965). The flavour of capric acid is described as fatty, soapy, waxy, coconut, burnt, cheese, harsh and oil (FlavorDB, accessed 27 September 2019). Apart from causing skin irritation in its pure form, myristic acid is not known to be allergenic.

[6] Equivalent to 9.9 mg L^{-1}

Potential for toxicity or allergenicity to people and toxicity to other desirable organisms from changes to the fatty acid profile

126. The highest concentration of medium chain fatty acids during cultivation of the GM *N. oceanica* would occur at harvest, when water is removed from the culture solution. Wet pastes of *Nannochloropsis* have a moisture content of approximately 65% (Chen et al., 2012). As shown earlier, the concentration of capric and lauric acids could reach 2% of dry weight in the microalgal pellet, with myristic acid potentially reaching 4% of dry weight. Thus, the concentration of fatty acids in a wet paste of GM *N. oceanica* could be up to 0.7% capric and lauric acids, and 1.4% myristic acid.

127. Routes of exposure have been identified earlier as direct contact with the microalgae or inhalation of aerosolised microalgae. Skin irritation due to capric, lauric or myristic acids at these concentrations is unlikely to occur if workers were exposed to the microalgal pellet during harvest (see *Properties of medium chain fatty acids* in this risk scenario). Inhalation of microalgae would only occur at very low quantities, as controls are required for this trial to minimise the likelihood of aerosolisation of GM *N. oceanica* during cultivation and harvest.

128. Capric, lauric and myristic acids are not regarded as allergens (see *Properties of medium chain fatty acids* in this risk scenario).

129. As discussed under *Causal pathway* in this risk scenario, other desirable organisms are not expected to come into contact with or ingest the GM *N. oceanica* during cultivation at the pilot plant. Thus, there is little potential for toxicity to desirable organisms under this risk scenario.

Conclusion

130. Risk scenario 1 is not identified as a substantive risk due to limited potential for exposure of people and other desirable organisms at the trial site; and the lack of toxicity or allergenicity of the introduced gene or its encoded protein and altered fatty acid profile to humans, and lack of toxicity to other desirable organisms that visit the trial site. 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 gene conferring increased production of fatty acids
Causal Pathway	⬇ Cultivation of *N. oceanica* expressing the introduced gene ⬇ Presence of GM *N. oceanica* outside the trial limits ⬇ Establishment and persistence of GM *N. oceanica* in a suitable aquatic environment ⬇
Potential Harm	Increased toxicity or allergenicity for humans OR Increased toxicity to other desirable organisms OR Reduced quality of the biotic environment OR Reduced establishment of desirable organisms

Risk source

131. The source of potential harm for this postulated risk scenario is the introduced gene for increased production of fatty acids in the GM *N. oceanica*.

Causal pathway

132. The GM *N. oceanica* is cultivated at the pilot plant and the thioesterase gene is expressed.

133. The GM *N. oceanica* could be present outside the trial limits, if it were dispersed off the trial site during the trial or if it persisted at the trial site after completion of the trial. If the latter occurred then the GM *N. oceanica* may be dispersed from the trial site at any time. In either case, the GM *N. oceanica* could establish and persist if it were dispersed into a suitable aquatic environment. This could increase the likelihood of exposure of people, other desirable organisms, or the environment to the introduced gene and protein, and to its increased or introduced fatty acids.

Potential for persistence at the trial site

134. The GM *N. oceanica* could persist at the trial site

- in culture vessels between batches of GM *N. oceanica* culture
- in other culture vessels, in which non-GM microalgae are grown while the GM *N. oceanica* is present at the trial site
- in equipment other than culture vessels used in connection with the GM *N. oceanica*
- in the waste water tank or
- in spills of culture solution suitable to grow marine microalgae.

135. Proposed controls would require both GM *N. oceanica* and all non-GM microalgal cultures grown at the site while GM *N. oceanica* is cultivated, to be grown in closed systems; to clean all equipment used in connection with the GM *N. oceanica* after use and before use with any other algae; and to regularly inspect the contents of the waste water tank for GM *N. oceanica*. These controls would minimise the likelihood of GM *N. oceanica* persisting undetected at the facility.

Potential for dispersal via aerosol formation

136. Baseline information on the biology of *N. oceanica* provides that the most likely means of dispersal of GM *N. oceanica* outside the trial site may occur through the release of airborne cells, resulting from the activities of people or animals, or through extreme weather events.

137. If aerosolised, microalgal cells can potentially travel long distances in the air and initiate new populations if deposited in a viable state in a suitable aquatic environment (Tesson et al., 2016; OGTR, 2019). Microalgae can become airborne by processes such as bubbles bursting, and sea spray forming through wind friction or breaking waves. It is unknown what percentage of *N. oceanica* might become airborne under standard cultivation conditions.

138. Cells of *N. oceanica* are approximately 2–5 µm in diameter, which is similar in size to fungal spores. Although the size of *N. oceanica* is similar to that of fungal spores, these microalgal cells are not adapted to dry environments and are not known to form resting or dispersal cells which are able to withstand harsh environmental conditions (OGTR, 2019). However, a study simulating fungal spore dispersal in a forest environment predicted that, if they become airborne, 20–86% of cells of this size would be dispersed beyond 2 km, but noted that the transport of spores does not equal colonisation (Norros et al., 2014). Modelling aerial dispersal of microorganisms with a diameter of 9 µm, Wilkinson et al. (2012) showed that, once airborne, a small proportion could travel between continents and stay airborne for several days.

139. Little is known about the ability of *Nannochloropsis* to survive once airborne. Data from the applicant shows that *N. oceanica* is not very tolerant of desiccation, with GM and wild type cells not surviving after being dried for 60 min at 25 °C (see Figure 6).

140. If GM *N. oceanica* dispersed off the trial site, it would need to be deposited soon into a suitable water body to survive, establish and persist. Experimental data supplied by the applicant shows that the GM *N. oceanica* can survive in Brisbane River water taken approximately 800 m from the proposed trial site. If GM *N. oceanica* reached the river, it could then be carried out to sea into a more suitable marine environment. However, maximum wind gusts near the proposed trial site have been recorded as originating mainly from a north-north-easterly, easterly and south-easterly direction which may limit dispersal of any airborne GM *N. oceanica* into a suitable aquatic environment (see Chapter 1, Figure 8).

141. As mentioned in Chapter 1, Section 6.2, an open pond field trial of GM microalgae of the species *Scenedesmus dimorphus* was conducted in the United States and included experiments to test the ability of GM *S. dimorphus* to disperse aerially at the field site (Szyjka et al., 2017). This species is a freshwater microalga in kingdom Plantae, approximately 10 µm in length. Genetically modified *S. dimorphus* were occasionally detected in the furthest traps, which were located 50 m from the open ponds.

142. Experimental data provided by the applicant shows that both GM- and non-GM *N. oceanica* do not grow in sterilised water taken from a freshwater creek. *Nannochloropsis oceanica* can survive and grow when transferred without adaptation to freshwater nutrient medium (Pal et al., 2013; Guo et al., 2018); however, it is unclear whether *N. oceanica* would survive transfer to rainwater or natural bodies of freshwater.

143. There is some concern in the literature that dispersal of GM microalgae during cultivation and harvest in open pond mass production systems would be inevitable (Henley et al., 2013; Beacham et al., 2017). It should be noted, however, that the proposed release would not be considered large scale, 'mass production' or 'open pond', and that Beacham et al. (2017) considered the use of glass houses and polythene tunnels as a reasonable level of containment. The proposed use of clear fitted plastic lids to cover the culture vessels could be considered an equivalent level of containment.

144. The proposed limits and controls, including fitting plastic lids to culture vessels at all times, would minimise the likelihood of dispersal via aerosol formation.

Dispersal through human activity

145. Dispersal through human activity could occur if staff did not adhere to protocols or by accident. For example, GM *N. oceanica* could be transported on clothing or footwear following a spill of GM *N. oceanica* cultivation solution.

146. Although human activity is a potential mechanism for microalgae dispersal from the pilot plant facility, the applicant has proposed limits and controls to prevent the dispersal of GM *N. oceanica* from the trial site. Access to the site would be restricted to authorised, trained staff. The site would need to be checked for spills of GM *N. oceanica* culture solution and action taken to destroy any spills of GMOs. All GM *N. oceanica* would be transported in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*, which would minimise the opportunity for dispersal of GM *N. oceanica* or for contact with any GM *N. oceanica* during transport between UQ and the trial site.

Dispersal by animals

147. Microalgae can be dispersed by animals, including birds and insects (Beacham et al., 2017). Rodents or large animals could cause damage to equipment used for the cultivation or harvest of the GM *N. oceanica*, leading to spills of the culture solution containing *N. oceanica*. The applicant monitored the presence of animals at the pilot plant facility over seven days using a motion-activated closed circuit television camera. Bird activity inside the fence and around the concrete pad was observed during the day. Three mice visited the pad during the night. Insect activity occurred mainly at night.

148. The applicant proposes to cover the culture vessels with clear plastic lids, which would prevent birds, insects and other animals from coming into contact with the GM *N. oceanica*. The culture bags used to contain GM *N. oceanica* inoculum would be hung in a manner that limits potential for spills and all tubing attached to the bags is secured. The pilot plant facility is surrounded by a fence to restrict entry by large animals, and the applicant proposes to manage rodents at the facility.

Dispersal in extreme weather

149. Extreme weather events, e.g. thunderstorms, hail, high winds or heavy rain, have the potential to disperse GM *N. oceanica* outside the trial location. The most likely means of spread is via wind or water. Dispersal via aerosol formation was discussed previously in this section. Dispersal by water could occur if it were able to flow into the culture vessels, which then may overflow. The applicant proposes to seal the culture vessels with clear hard plastic lids during cultivation, which are designed to prevent dispersal by

wind. The lids also prevent rainwater and hail from entering the vessels. Additionally, the concrete pad on which the culture vessels are located is surrounded with a lip to retain any overflowing water or culture solution on the concrete pad for decontamination.

Ability of GM Nannochloropsis oceanica *to establish outside trial limits*

150. Should GM *N. oceanica* be dispersed outside the trial, then it would need to establish in a suitable aquatic environment. Expression of the introduced thioesterase gene and alteration of the fatty acid profile in the GM *N. oceanica* may reduce the stability of the membranes of the GM *N. oceanica*; or change the palatability or nutritional value of the GM *N. oceanica* compared with non-GM *N. oceanica* (Mitra and Flynn, 2005; Flynn et al., 2013).

151. Experimental data provided by the applicant shows that the GM *N. oceanica* have a similar or better ability to grow in sterilised river water and seawater than non-GM *N. oceanica* (Chapter 1, Section 4.4). Growth of GM and non-GM *N. oceanica* was shown to be better in seawater than in brackish river water, as would be expected of a marine microorganism. Thus, if GM *N. oceanica* were to land in the Brisbane River, approximately 800 m from the trial site, it would be unlikely to be competitive with other microorganisms in their native environment. If GM *N. oceanica* were to reach a marine environment, it is possible that they could establish at a similar density as non-GM *N. oceanica*.

152. In another GM microalgae, *Dunaliella tertiolecta*, also modified to produce fatty acids with medium chain length, production of neutral lipids was halved when compared with its non-GM parent (Lin and Lee, 2017). Fatty acids of all lengths are used in membrane lipids, with fatty acids with medium chain length being edited out from the membrane lipids and channelled to proper storage lipids. Thus the altered proportions of fatty acids produced in the GM *N. oceanica* may affect the structural integrity of the membranes and be detrimental to the cell (Lin and Lee, 2017). If this were the case in the GM *N. oceanica*, then it would have no advantage over related organisms in the environment. However, since the growth of non-GM and GM *N. oceanica* was similar in Brisbane River water and seawater, the altered fatty acid profile does not seem to have had an effect on the membrane stability of the GM *N. oceanica*.

Potential Harm

153. If the GM *N. oceanica* were present outside the trial limits, then it may cause harm to people or the environment. If the GM *N. oceanica* were dispersed into a suitable aquatic environment, and established and persisted, then it may cause increased toxicity or allergenicity in people; increased toxicity to other aquatic organisms, or outcompete other desirable organisms, e.g. by having decreased palatability or by having another advantage in the environment. If GM *N. oceanica* were prone to dominate the environment, then it may form harmful algal blooms resulting in a reduced quality of the biotic environment or in reduced establishment of desirable organisms.

154. Risk Scenario 1 found that there is no reasonable expectation that the introduced thioesterase gene and protein would be toxic or allergenic in people, or toxic to organisms other than people. It is not expected that this conclusion would be different for Risk Scenario 2.

155. Therefore, this discussion will focus on the potential harms, which could occur as a result of the increased concentration of medium chain fatty acids present in the GM *N. oceanica*.

Presence of medium chain fatty acids in the environment

156. Capric, lauric and myristic acids are naturally present at low concentrations in marine algae (McCauley et al., 2015), and in microalgae that grow in freshwater and brackish environments (Thao et al., 2017).

157. Fatty acids with medium chain length are rarely present or occur in low concentrations in vegetable oils. A notable exception is coconut oil, in which lauric acid comprises almost half of total fatty acids, and capric and myristic acids are present at approximately 5% and 7%, respectively (Bhatnagar et al., 2009; Orsavova et al., 2015). Coconut oil has a long history of safe use in food. Fatty acids are not known coconut allergens (Anagnostou, 2017). In mammals, capric, lauric and myristic acids are present in milk, e.g. up to

2.4%, 13.8% and 12.1% of total fatty acids, respectively, in the breast milk of women (Yuhas et al., 2006) and at approximately 5.1%, 1.9% and 4.6%, respectively, in the milk of goats (Sumarmono, 2015).

158. Organic compounds, including fatty acids, are secreted by microalgae into surrounding water. Under favourable conditions, Nalewajko (1966) reported that a selection of microalgae excreted less than 2% of carbon fixed during photosynthesis; however, secretion can increase considerably in response to various stresses. Release of fatty acids from microalgae also occurs when the cell is damaged by a pathogen or predator (Desbois and Smith, 2010).

159. Free fatty acids have antimicrobial properties and are used as a defence mechanism by many organisms, including algae and other aquatic organisms, plants, animals and humans (Desbois and Smith, 2010). Antimicrobial properties of saturated fatty acids vary with chain length. Some researchers have found that capric and lauric acids have greater antimicrobial effect than other chain lengths, while other researchers find that myristic, palmitic (C16:0) and stearic (C18:0) acids have greater activity (Proctor, 1957; Desbois and Smith, 2010, and references therein).

Potential for toxicity or allergenicity to people and toxicity to other desirable organisms from changes to the fatty acid profile

160. Risk scenario 1 found that there is little potential for toxicity or allergenicity to humans from exposure to the GM *N. oceanica* microalgal biomass at harvest. This concentration of GM *N. oceanica* cells would not be attained if GM *N. oceanica* established outside the trial limits, including if it were to grow to algal bloom proportions. Thus, there is a lack of potential for toxicity or allergenicity to humans from exposure to the GM *N. oceanica* in this Risk scenario.

161. If GM *N. oceanica* established in a suitable water body, desirable aquatic organisms would be exposed to the GMO. Exposure to the medium chain fatty acids produced by the GMO would be greatest to predators and pathogens that directly interact with *N. oceanica* and cause cell lysis. *Nannochloropsis oceanica* is at the bottom of the food web, and predated by various types of zooplankton (OGTR, 2019). Other desirable organisms might be exposed incidentally to the fatty acids if they were present in the seawater surrounding the GMOs.

162. As discussed in Risk scenario 1, the concentration of capric acid and lauric acid in the GM microalgal cell could reach approximately 0.7% of wet weight, which is equivalent to a concentration of 7 mg L^{-1}. Myristic acid could reach 1.4% of wet weight, which is equivalent to 14 mg L^{-1}. Little information is available on toxic effects of these fatty acids in grazers of microalgae. However, some toxicity information is available for other fresh or marine water organisms (Table 2). The freshwater crustacean *Daphnia magna* is affected when held in solutions containing 16 mg L^{-1} capric acid (immobilisation, EC_{50}). The median lethal concentration (LC_{50}) for brine shrimp is 36 mg L^{-1} capric acid in solution. The no observed effect concentration (NOEC) for the freshwater fish *Danio rerio* ranges from 2–23.6 mg L^{-1} capric acid in solution. However, the effect of ingesting microalgal cells containing this concentration of capric acid as part of a mixture of different phytoplankton is unknown and is an area of uncertainty for this risk assessment.

163. Cell lysis can release the medium chain fatty acids into solution. Cell lysis occurs, e.g., during microalgal blooms, as a result of viral infections (Fuhrman, 1999). An assessment of the environmental fate of capric acid in water reports that the fatty acid is expected to adsorb to suspended solids and sediment (PubChem Hazardous Substances DataBank website, accessed 9 January 2020). The assessment also noted that biodegradation may be an important process, citing a study by Kondo et al. (1988) that found capric acid to be easily biodegradable in river and seawater.

164. Cell densities recorded for a bloom of *N. granulata* off the coast of China were 10^9–10^{10} cells L^{-1} (Zhang et al., 2015). If GM *N. oceanica* reached these densities and were lysed, the concentration of capric acid could reach 1.2 mg L^{-1} in solution. As biodegradation and dilution of any fatty acids would occur rapidly in the ocean, only acute (short term) toxicity studies are relevant. At this concentration, capric acid has a toxic effect on biomass production in the freshwater microalga *Scenedesmus subspicatus* (Table 2). This concentration is below the reported concentrations for growth inhibition (GI50) for six other microalgal species shown in Table 2; highest NOECs for these species is unknown.

165. The range of cell densities of *N. oceanica* in its natural habitat is not known; however, in a study of microalgal species in coastal waters on the east coast of the United States, Oseji et al. (2019) reported that the most abundant ochrophyte, *Chattonella* sp., reached a density of approximately 10^4 cells L^{-1}. If cell lysis released the contents of GM *N. oceanica* growing at this density into water, the concentration of capric acid would be approximately 1 ng L^{-1}, which is well below toxic concentrations for aquatic organisms.

166. The total lipid content of GM *N. oceanica* is expected to be similar to non-GM *N. oceanica*. The increase in medium chain fatty acids is expected to be linked to a decrease in palmitic acid (C16:0, Figure 7). Comparison of toxicity data for capric, lauric, myristic and palmitic acids shows that the toxicity of these fatty acids to aquatic organisms is similar (Table 4). Thus, it is unlikely that there would be a significant increase in toxicity of the GM *N. oceanica* as a result of the altered fatty acid profile, compared with non-GM *N. oceanica.*

Table 4 Comparison of toxicity data for capric, lauric, myristic and palmitic acids for some aquatic species.

Organism	Fatty acid	Toxicity (mg L^{-1})	Comments[a]
Microalgae (*Pseudokirchneriella subcapitata*)	Capric acid	15	EC_{50} (growth rate), 72 h, static
	Lauric acid	>7.6	
	Myristic acid	>2.1	
	Palmitic acid	>0.9	
Microalgae (*Pseudokirchneriella subcapitata*)	Capric acid	1.8	NOEC (biomass), 72 h, static
	Lauric acid	4.4	
	Myristic acid	2.1	
	Palmitic acid	>0.9	
Daphnids (*Daphnia magna*)	Capric acid	0.2	NOEC, 21 d, semi-static
	Lauric acid	0.47	
	Myristic acid	0.31	
	Palmitic acid	0.22	

EC_{50}, median effective concentration; NOEC, no observed effect concentration. Note that data for palmitic acid is limited, because solubility in water decreases as fatty acid chain lengths increase.
Source: European Chemicals Agency website (accessed 11 December 2019).

Potential for reduced quality of the biotic environment or reduced establishment of desirable organisms from changes to the fatty acid profile

167. Reduced quality of the biotic environment or reduced establishment of desirable organisms could occur if GM *N. oceanica* outcompeted other phytoplankton and the cell density increased to bloom concentrations, or if the GMO was more capable of accumulating pollutants than non-GM *N. oceanica*.

168. Microalgae optimised for biofuel production, i.e. producing shorter saturated fatty acids, are expected to be less palatable to some predators than their comparators, due to altered nutritional value (Flynn et al., 2013). The stoichiometric ratio of carbon, nitrogen and phosphorus is a measure of nutritional value. Microalgae with a higher ratio of carbon are considered less palatable (Flynn et al., 2013). The total lipid content of the GM *N. oceanica* is not expected to change. As fatty acids are made up of carbon, hydrogen and oxygen atoms, the stoichiometric ratio of carbon to nitrogen and phosphorous should not differ between the GM *N. oceanica* and non-GM *N. oceanica.*

169. Long chain polyunsaturated fatty acids are important nutritional components of phytoplankton for copepod grazers (Wichard et al., 2007). It is anticipated that the increase in saturated medium chain fatty acids would be offset by a decrease in the saturated long chain fatty acid, palmitic acid (C16:0, Figure 7).

The concentration of polyunsaturated and unsaturated fatty acids is not expected to differ greatly between the GM *N. oceanica* and non-GM *N. oceanica*.

170. Copepods grazers, the major consumers of marine phytoplankton, reject prey that produce toxic compounds (Huntley et al., 1986). As discussed earlier, the concentration of capric acid within GM *N. oceanica* cells could reach up to 7 mg L^{-1}. This value is only slightly lower than long-term LC_{50} value of 9.8 mg L^{-1} in solution for zebrafish, and the immobilisation EC_{50} of 16 mg L^{-1} in solution for daphnids (Table 2). It is possible that the GM *N. oceanica* could be rejected by some grazers and that this could lead to an increase in number of the GMOs compared with non-GM *N. oceanica.* A search of the literature did not reveal any toxicity data for capric acid in copepods. This is an area of uncertainty for this risk assessment. There have been no studies reported, which investigate the palatability of GM *N. oceanica* compared with the non-GM *N. oceanica*. Similarly, there are no studies on the GM *N. oceanica* investigating whether it may have an advantage in the environment, such as increased fitness. These are areas of uncertainty for this risk assessment. However, it is noteworthy that the growth characteristics of the GMOs and non-GM *N. oceanica* are very similar.

171. Algal blooms require a number of abiotic and biotic circumstances to coincide for them to eventuate. Important abiotic factors include the availability of nutrients, especially nitrates and phosphates, ideal water temperatures, and little mixing within the water column. Numerous biotic interactions also play a role. However, many algal blooms form when the density of phytoplankton grazers is reduced through over-predation by higher trophy organisms rather than through grazers either not eating the bloom forming algae or being affected by algal toxins (e.g. reviewed in Turner and Granéli, 2006). This means that non-GM *N. oceanica* could have formed occasional algal blooms if it were capable of doing so. However, as discussed in Chapter 1 (Section 3) and in *The Biology of* Nannochloropsis oceanica *Suda & Miyashita (a microalga)* (OGTR, 2019), non-GM *N. oceanica* is not known to form harmful algal blooms.

172. The change in fatty acid profile of the GM *N. oceanica* could alter the rate of bioaccumulation of environmental pollutants, particularly lipid soluble compounds such as polychlorinated biphenyls (PCBs). Studies have shown that PCB uptake in microalgae increases with increased lipid content, e.g. Lynn et al. (2007) and references therein. The lipid content of the GM *N. oceanica* is not expected to increase, compared with non-GM *N. oceanica*; however, the effect of an altered fatty acid profile on the uptake of pollutants is unclear, and, therefore, another area of uncertainty for this risk assessment.

Conclusion

173. Risk scenario 2 is not identified as a substantive risk due to the limited ability of the GM *N. oceanica* to establish and persist outside of cultivation at a density that would have an appreciable adverse effect on people, desirable organisms or the environment. Therefore, this risk could not be considered greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

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

175. 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.

176. As trials of GMOs are designed to gather data, there are generally data gaps when assessing the risks of a trial application. However, 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.

177. For DIR 169, uncertainty is noted particularly in relation to:

- potential for increased toxicity and allergenicity of GM *N. oceanica* to people or desirable organisms

- the effect of the genetic modification on total fatty acid production
- potential for increased spread and persistence, leading to reduced quality of the biotic environment and/or reduced establishment of desirable organisms.

178. 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.

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

Section 4 Risk evaluation

180. 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.

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

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

182. Two risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the control measures proposed by the applicant, and considering both the short and long term, none of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 3 and include:

- the introduced gene, its expressed protein and the fatty acids produced are unlikely to be toxic or allergenic at concentrations present in the GM *N. oceanica*
- no GM *N. oceanica* would enter human food or animal feed
- limits on the size and duration of the proposed release
- suitability of proposed controls to restrict the spread and persistence of the GM *N. oceanica* and its genetic material.

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

Chapter 3 Risk management plan

Section 1 Background

183. 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.

184. Under Section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment.

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

186. 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

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

Section 3 General risk management

188. 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 proposed size, location and duration of the release, and to restrict the spread and persistence of the GMOs and their genetic material in the environment. The conditions are discussed and summarised in this chapter and listed in full in the licence.

3.1 Licence conditions to limit and control the release

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

3.1.1 Consideration of limits proposed by UQ

190. The proposed release would take place at a single location at UQ's Pinjarra Hills campus in Qld, which is owned and managed by UQ. The trial would run over several periods totalling up to 12 months. Genetically modified *N. oceanica* would be cultivated in up to six culture vessels, each with a cultivation volume of up to 600 litres.

191. In the initial stages of the application, the applicant requested the release to take place until December 2022. The applicant subsequently requested an increase to the licence period to December 2023 to allow extra time for approval and preparation of lids for the culture vessels, which is expected to take around six months. The increase in duration of the trial is not considered to affect the conclusion of the Risk Assessment, as the total cultivation period remains unchanged. The limited size and duration of the trial restricts the potential exposure of people and desirable animals to the GMOs (Risk scenario 1).

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

3.1.2 Consideration of proposed controls to manage exposure to the GMOs

193. The applicant proposed not allowing the GMOs or GM products to be used for human food or animal feed, and all waste from the trial would be disposed of in a manner that would not disperse GMOs. This condition restricts the exposure of people and desirable animals to the GMOs (Risk Scenario 1).

194. The applicant proposed the fence surrounding the site as a control to exclude unauthorised people and large animals from accessing the trial site. Large animals entering the trial site could damage equipment and possibly disperse GM *N. oceanica*, but are not expected to ingest the microalgal solution (Risk Scenario 1). Therefore, the licence requires that large animals be excluded from the site, although it is not prescriptive as to the method used to achieve this outcome. This condition restricts the exposure of people not authorised under the licence and of desirable animals to the GMOs (Risk Scenario 1).

3.1.3 Consideration of proposed controls to manage persistence of the GMOs

195. After each harvest, the applicant proposed to decontaminate culture vessels and equipment to ensure that GM *N. oceanica* do not remain after harvest. The applicant also proposed to destroy any material from the cultivation trials that is not kept for evaluation or storage. Licence conditions require that the culture vessels and equipment must be decontaminated (which would destroy any viable GM microalgae) as soon as practicable after harvest, and that harvested GM *N. oceanica* not required to conduct experiments or for future cultivation, must be destroyed as soon as practicable. A condition also states that culture vessels and equipment in contact with the GM *N. oceanica* must not be used for any other purpose until they have been cleaned.

196. The applicant proposed that any non-GM microalgae cultivated at the pilot plant, while GMOs are being cultivated, would be treated as though they were GMOs. The GM *N. oceanica* could potentially be dispersed into the non-GM microalgal cultures grown at the site, and this could lead to persistence of the GMOs. Therefore, a licence condition states that, during cultivation of GM *N. oceanica* at the site, non-GM microalgae grown at the site must be grown in closed systems. In the unlikely event that GM microalgae is released during cultivation and settles in open empty vessels located at the site, the standard practice of decontaminating vessels before cultivating microalgae will reduce the likelihood of any GMOs being unintentionally propagated during cultivation of non-GM microalgae. This has been imposed as a licence condition.

197. To ensure that the GMOs are not accidently propagated in non-GM microalgae samples taken off-site, the licence does not permit non-GM microalgae grown at the site between the start of cultivation of the GMOs until site sign-off to be used as an inoculum for further cultures of microalgae. There is also a

condition in the licence requiring that the GM *N. oceanica* must be harvested separately from any non-GM microalgae.

198. The applicant proposed that GM *N. oceanica* would be destroyed as necessary and after completion of the trial using chemical treatment (such as bleach, herbicide or 80% ethanol), destructive analysis or autoclaving. These methods are considered effective in destroying microalgae, and are included as methods of destruction in the licence.

199. Following harvest, the applicant proposed monitoring the waste water tank for the presence of GM *N. oceanica* at least quarter yearly for at least 12 months, and until the last six months are free of the GM *N. oceanica*. If GM *N. oceanica* are detected in the waste water tank, they would be destroyed. Following consultation, the requirements for post-decontamination monitoring of the site were amended. The licence holder must demonstrate that all equipment and areas requiring decontamination, including the waste water tank, are free from GM *N. oceanica.* Ongoing monitoring is not required as there is little evidence that *N. oceanica* would form survival structures (OGTR, 2019), and it is unlikely that viable GM *N. oceanica* would persist in culture vessels or on equipment following cleaning.

200. The GM *N. oceanica* are unable to use nitrates as a nitrogen source, due to the partial deletion of two genes necessary for the transport and metabolism of nitrates (Chapter 1, Section 4.1). Nitrate is generally the most abundant form of nitrogen in the Brisbane River and Moreton Bay, with ammonia and nitrite often present at lower concentrations (Chapter 1, Section 5.1). The inability to use nitrate was proposed by the applicant as a control to reduce the competitiveness of the GM *N. oceanica* in the natural environment, compared with non-GM *N. oceanica.* Experimental data provided by the applicant showed that growth characteristics of GM and non-GM *N. oceanica* are similar; however, there was not sufficient evidence of reduced fitness. Thus, although the inability to use nitrates is considered an appropriate control to reduce likelihood of persistence of the GMOs, this trait has not been considered as a control for the purpose of conducting the risk assessment.

3.1.4 Consideration of proposed controls to manage dispersal of the GMOs

201. The applicant proposed control measures to limit the dispersal of GM *N. oceanica* during cultivation. The applicant proposed fitting clear plastic lids over the culture vessels to minimise the likelihood of aerosol release and subsequent dispersal by wind, people or animals. Sealable inlet and outlet ports inserted into the lids are proposed to allow inoculation of cultures, insertion of probes and cooling loops, and supply and release of gases; these ports could be filtered with a cell-free filter if required. The applicant also stated that equipment would be decontaminated after contact with the GM *N. oceanica* solution. These controls are considered appropriate to minimise the likelihood of dispersal of the GM *N. oceanica* and are covered in several licence conditions. However, as the lids have not yet been made and because the lids are an important measure to limit dispersal of the GMOs, the Regulator imposed a condition requiring approval of the lids once they have been manufactured. In addition, outlet air would need to be decontaminated to minimise dispersal of aerosolised GMOs, and a licence condition requires approval by the Regulator of a method to achieve this.

202. The applicant proposed physical and behavioural control measures to limit the dispersal of GM *N. oceanica* during harvest, by minimising aerosol formation. Genetically modified *N. oceanica* are harvested by pumping the culture solution from the culture vessel to the centrifuge. The applicant proposed placing containers under connection points (e.g. under the culture vessel connector/pipe and pipe/pump connections) to collect spillages; spilled culture would be treated with hypochlorite prior to disposal. As mentioned previously, lids would remain on the culture vessels at all times until they have been cleaned. These controls are considered appropriate to minimise the likelihood of dispersal of the GM *N. oceanica* and are covered in the licence conditions.

203. A further physical control measure present at the proposed facility is a bund around the periphery of the concrete pad to ensure spill containment and prevent runoff of GM *N. oceanica* into the environment in case of a spill. Although the GM *N. oceanica* have not been found to pose risks to the health and safety of people, or to the environment (Risk Scenarios 1 and 2), dissemination of the GMOs must be restricted under a limited and controlled release licence. If the GM *N. oceanica* were to be dispersed off the concrete

pad in runoff water, the licence holder would have limited ability to control the GMOs. The bund was originally 150 mm high and designed to capture up to 90% of all local predicted rainfall based on ten years of weather data. After a renovation of the pad, the bund is now approximately 250 mm high and expected to retain all rainfall with the possible exception of the most extreme rainfall events. The applicant also proposed to install bunds around individual culture vessels, if required. A bund surrounding the concrete pad is considered sufficient to capture any spilled GM *N. oceanica* solution and prevent dispersal in runoff water, and is included as a licence condition. The licence also requires that all rainfall and any other water on the concrete pad, and any used microalgal culture solution, must be collected in a waste water tank and treated in a manner that will destroy any GM *N. oceanica*, before any water may be released from the waste water tank into the environment.

204. The applicant stated that standard operating procedures can be put in place to limit the likelihood of dispersal in the event of forecast extreme weather. A two-hour response time is required to organise transport and travel to the site. It is expected that the securely fitting plastic lids would prevent dispersal of GM *N. oceanica* via wind or via rain entering culture vessels and causing flooding; the use of these lids is required by the licence. A further condition in the licence requires that the licence holder notify the Regulator when any extreme weather event is expected to affect or has already affected an area where the GMOs are or may be present.

205. The applicant proposed the cultivation of a marine species of microalgae in a location surrounded by freshwater water bodies as a control measure. Experimental data supplied by the applicant indicates that any GM *N. oceanica* that were to disperse to such a water body, such as a freshwater creek or lake, would be unlikely to persist (Chapter 1, Section 4.4). The proposed pilot plant facility is located approximately 800 m from the nearest water body with sufficient salinity to allow *N. oceanica* growth (Brisbane River; Chapter 1, Section 5.1). The nearest non-permanent drainage feature which flows into the Brisbane River is approximately 100 m from the facility, and a freshwater lake is approximately 170 m distant. Other open ponds suitable for *N. oceanica* growth are located on the Pinjarra Hills campus at a distance of approximately 1.3 km from the facility (Chapter 1, Section 5.4). The location of the proposed facility is considered suitable to minimise the likelihood of establishment of the GM *N. oceanica* off-site (Risk Scenario 2), thus no specific licence condition is required. The Act requires that licence holders inform the Regulator if they become aware of additional information as to any risks to the health and safety of people, or to the environment. This would include any change in the distance between the facility and water suitable for *N. oceanica* growth, e.g. if open microalgal culture vessels/ponds were to be built near the facility.

206. The applicant has stated that rodent control is in place at the proposed facility. Rodent activity has been detected at the facility at night (Chapter 1, Section 5.2). Rodents could damage equipment, which could lead to the dispersal of GM *N. oceanica* (Risk Scenario 2). A licence condition states that, while the GM *N. oceanica* are being cultivated, rodents must be controlled at the site and the facility must be maintained in a manner that does not attract or harbour rodents.

207. Birds have been detected at the facility during the day (Chapter 1, Section 5.2). Birds could potentially disperse microalgae in water droplets on feathers, if they came into contact with microalgal solution (Risk scenario 2). The licence conditions (including fitting lids to culture vessels, and cleaning up any spilled microalgal solution) minimise the likelihood of birds coming into contact with and dispersing the GM microalgae. Thus, no specific licence conditions related to birds are imposed.

208. The applicant proposed that equipment would be cleaned before using it for any other purpose. This is considered appropriate to ensure GM *N. oceanica* are not unintentionally dispersed by equipment. Thus, the licence contains a condition that requires any equipment used in connection with the GMOs to be decontaminated as soon as practicable after use and before use for any other purpose. If equipment is not decontaminated immediately after use, it is required to be stored in a manner that avoids dispersal of GM *N. oceanica*. Decontamination of equipment associated with transport and storage of the GMOs needs to be conducted according to the requirements set out in the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*.

209. The applicant proposed that any GM *N. oceanica* would be transported, via the shortest practical route, to approved facilities according to the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. Any GM *N. oceanica* would be stored in approved facilities. Standard conditions in the licence require transport and storage of GMOs in a manner that minimises exposure of people and other desirable organisms to the GMOs (Risk Scenario 1) and dispersal into the environment (Risk Scenario 2).

3.1.5 Additional control to manage dispersal of the GMOs

210. Further details about the proposed process of cultivating the GM *N. oceanica* were provided by the applicant during consultation. Plastic culture bags containing the GM *N. oceanica* would be suspended outside on the pilot plant for a full day and night, in order for microalgae to acclimatise to outdoor conditions prior to inoculation into the culture vessels. The applicant stated that this technique of adapting cultures to natural conditions has been used regularly for all outdoor growth experiments since the pilot plant started operating in 2013, e.g. Wolf et al. (2016), and that they have never had issues with either leaks or animals damaging the bags. A licence condition requires that the bags are protected from damage and that the bags are not hung outside on the pilot plant for more than 48 hours.

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

211. 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 a maximum of three years, until December 2023
- limit the release to a single location in Qld: UQ's Centre for Solar Biotechnology pilot plant facility, Pinjarra Hills campus
- limit the trial size to a maximum volume of six 600-litre culture vessels per experiment
- securely fit lids to culture vessels until post-harvest cleaning is completed
- exclude large animals from the site
- implement measures to control rodents within the site
- capture and treat all rainfall and any other water on the concrete pad of the site during cultivation of the GM *N. oceanica*
- inspect the site for spilled culture medium regularly during cultivation of the GM *N. oceanica*
- cultivate and harvest GM *N. oceanica* in a manner that avoids dispersal of the GMOs
- harvest the GM *N. oceanica* separately from any non-GM microalgae
- clean areas and equipment exposed to GM *N. oceanica* after use and before use for any other purpose
- destroy all GMOs not required for further experiments or future trials
- treat water in the waste water tank in a manner that will destroy any GM *N. oceanica*, prior to release of water from the tank
- transport and store the GMOs in accordance with the Regulator's guidelines
- not allow the GM *N. oceanica* to be used for human food or animal feed.

3.2 Other risk management considerations

212. 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

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

- any relevant convictions of the applicant
- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- the capacity of the applicant to meet the conditions of the licence.

214. The conditions of the licence include a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

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

3.2.2 Contingency plan

216. The University of Queensland is required to submit a contingency plan to the Regulator before cultivating the GMOs. This plan must detail measures to be undertaken in the event of any unintended presence of the GM microalgae outside permitted areas.

217. Before cultivating the GMOs, UQ is also required to provide the Regulator with a method to detect the GMOs reliably and uniquely.

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

218. 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 cultivating the GMOs, UQ 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

219. 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 trial
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the trial.

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

- expected and actual dates of cultivation
- details of cultivation volumes
- expected and actual dates of harvest and cleaning after harvest
- details of inspection activities.

3.2.5 Monitoring for compliance

221. 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 site.

222. 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.

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

Section 4 Issues to be addressed for future releases

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

- additional molecular and biochemical characterisation of the GM *N. oceanica* (including fatty acid composition), particularly with respect to potential for increased toxicity and allergenicity
- additional studies to test the ability of the GM *N. oceanica* to compete with organisms in the environment or cause harm.

Section 5 Conclusions of the RARMP

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

226. Licence conditions have been imposed to limit the proposed size, location and duration of the release, 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

Advice received by the Regulator from prescribed experts, agencies and authorities[7] on the consultation RARMP is summarised below. All issues raised in submissions that related to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence and were used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Submission	Issues raised	Comment
1	Agrees with the overall conclusions of the RARMP.	Noted.
	The Regulator should further consider the requirements for containing the GMOs to the trial site.	Licence conditions have been revised and strengthened to limit the potential for dispersal of the GMOs from the trial site.
	The Regulator should further consider additional phenotypic information for the GMO and whether there is potential for adverse human health and environmental effects.	Additional information about the altered fatty acid profile was requested from the applicant and included in the RARMP at paragraph 118. Further discussion was included in *Risk Scenarios 1 and 2* of the potential for adverse human health and environmental effects.
2	Is broadly supportive of application DIR 169, particularly as it has been noted that the RARMP has not identified a significant risk to human health and safety, or the environment for the proposed release of GM microalgae. It was noted that further work will need to be done on the safety as a food source and proliferation potential in the marine environment, once commercial potential has been established. Indicated that the licence conditions looked adequate and that they are interested in the results of the trial.	Noted.
3	Considered the RARMP and did not have any concerns. Pointed out that the location was affected by drought, which would limit the ability of the GMOs to establish.	The potential for dispersal of the GMOs beyond the pilot plant facility at Pinjarra Hills was considered in *Risk Scenario 2*. No substantive risks to human health and safety or to the environment were identified as a result of the genetic modification.

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

4	Reports that overall the application has negligible risks to the health and safety of people and the environment. Is satisfied that proposed measures to manage the short and long term risks of the application are adequate.	Noted.
5	Agrees with the overall conclusions of the RARMP that the risks to the environment are negligible due to the controls in place that minimise any dispersal or exposure to the GMO.	Noted.
	Considers that the RARMP would benefit from additional discussion regarding the toxicity of the fatty acids to organisms other than humans.	Additional toxicity data was included in Table 2 and in *Risk Scenario 2*. The assessment of toxicity data was revised to include further endpoint data for aquatic organisms. Risk scenario wording was edited to clarify that *Risk Scenario 1* assesses the toxicity of fatty acids to organisms if they are exposed at the trial site, while *Risk Scenario 2* assesses toxicity to organisms that might be exposed outside the trial limits.
	Advises that more information should be included on whether the GMO could survive, persist or increase in abundance compared to its non-GM counterpart, e.g. clarification on the effectiveness of genetic modifications intended to reduce fitness such as inability to use nitrate as a nitrogen source.	The effectiveness of genetic modifications intended to reduce fitness is an area of uncertainty for this application. Data provided by the applicant showed that the GM and non-GM *N. oceanica* had similar growth characteristics. Therefore, this trait (inability to use nitrate) was not considered as a control for the purposes of the RARMP. For clarity, *Risk Scenario 2* was edited to remove reference to potential fitness reduction.
	Advises to include additional discussion on competitiveness and persistence in marine environments that may lead to potential adverse impacts on biota.	Additional discussion was included on the potential for reduced predation by zooplankton as a result of the altered fatty acid profile.
	Recommends for future research before any larger-scale trials to find out about fatty acid toxicity to aquatic organisms, including exposure pathways; potential reduced predation patterns; and increased abundance, survival, and competitiveness. Noted that uncertainty could be addressed in future releases by investigating whether the GMOs are able to compete and survive in non-sterilised water from natural aquatic environments; whether the inability of the GMO to use nitrate is an effective method to	Noted. Additionally information for future releases is listed in the RARMP, including molecular and biochemical characterisation of the GM *N. oceanica* (including fatty acid composition), particularly with respect to potential for increased toxicity and allergenicity, and studies to test the ability of the GM *N. oceanica* to compete with organisms in the environment or cause harm.

	limit its survival or persistence; whether there are other effective methods to limit the GMO's ability to survive, and whether zooplankton prefers to predate on the GM or non-GM *N. oceanica*.	
6	Considered the RARMP and undertook to seek comments externally from a subject matter expert. Overall, believes that the proposed licence conditions and control measures are adequate to deal with issues that may arise on this limited and controlled GMO release and supports the Regulator's conclusion that DIR 169 poses negligible risk of harm to human health and safety and the environment.	Noted.
	Noted that the controls proposed by the applicant (page 2-point 2.2) to restrict the spread and persistence of the GMOs in the environment is not adequate to stop the spread of the algae.	The risks from the trial were assessed in Chapter 2 (Risk Assessment), and no substantive risk identified. The appropriateness of the controls proposed by the applicant in Chapter 1, Section 2.2 are discussed in Chapter 3 (Risk Management Plan). Additional controls have been included as licence conditions to limit the spread of the microalgae.

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

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

Submission	Issues raised	Comment
1	I object to the above development because I do not believe that advances in biotechnology can help or save us from the ecological collapse happening now, caused by over-development and technological innovation.	The Regulator must consider risks to human health and safety and to the environment posed by genetic modification being assessed in the application. No specific risks from this application are raised in the submission.