Australian Government
Department of Health and Aged Care
Office of the Gene Technology Regulator

November 2023

Summary of the Risk Assessment and Risk Management Plan (consultation Version)

for

Licence Application No. DIR 200

Introduction

The Gene Technology Regulator (the Regulator) has received a licence application to use genetically modified (GM) *Pichia pastoris* (yeast) for precision fermentation to produce bovine milk, chicken egg and spider silk fibre proteins. It qualifies as Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment under the *Gene Technology Act 2000* (the Act).

The applicant, Cauldron Molecules Pty Ltd (Cauldron) proposes to produce a range of recombinant proteins using various strains of *Komagataella phaffii* (previously known as *Pichia pastoris*[1]). *P. pastoris* is a non-pathogenic yeast that is widely used in the biotechnology industry to produce recombinant proteins for pharmaceutical or food enzyme use. The proposed application is to optimise the large-scale fermentation process and characterise GM yeast used to produce recombinant animal proteins from a non-animal source. The GM yeast will incorporate a protein expression cassette to produce a recombinant protein. The incorporated genes will encode for proteins in their native form. The production process will involve fermentation of GM yeast cultures in large volumes (approximately 12,500 L per tank) at Cauldron's purpose-built protein production facility in Borenore, New South Wales. The recombinant proteins will be purified and will not contain any GM yeast.

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. Licence conditions have been drafted for the proposed trial. The Regulator invites submissions on the RARMP, including draft licence conditions, to inform the decision on whether to issue a licence.

[1] Host name *Pichia pastoris* will be used for this application.

The application

Project Title	Fermentation and processing of recombinant proteins using genetically modified *Pichia pastoris*[2]
Parent organism	*Pichia pastoris*
Genetic modifications	Insertion of expression cassette for producing bovine milk, chicken egg and spider silk fibre proteins. The expression cassette may also contain: • antibiotic selectable marker gene that confers resistance to a specific antibiotic to enable selection for the GM yeast • secretion signal peptide to facilitate secretion of proteins • constitutive or inducible promoter to facilitate expression of introduced sequences • tags such as epitope or polyhistidine to detect and purify the recombinant proteins
Principal purpose	To optimise the fermentation process and characterise GM yeast during production of animal proteins.
Previous releases	There has been no previous application in Australia for these GMOs.
Proposed limits and controls	
Proposed duration	5 years
Proposed location	Cauldron facility in Borenore
Proposed controls	• Laboratory strains of yeast will be used in the production which require specific media and growth conditions. • Molecular characterisation for multiple generations will be undertaken to assess genetic stability and copy number. • GM yeast will be fully transformed, i.e. vector plasmids will not be present. • Fermentation will take place in closed systems and transfer of fluids will be aseptic. • Transport of viable GM yeast will follow the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. • Viable GM yeast will be decontaminated via steam sterilisation or chemical disinfection. • Staff handling the GM yeast will undergo licence specific training. • Stringent manufacturing practices and quality control procedures will be followed to ensure that GM yeast does not remain in the environment. • The production facility is located in a region where there are no oak or chestnut trees in the proximity, which are the sources of wild-type *P. pastoris*. • Only inactivated GM yeast slurry may be used for animal feed preparations or as soil conditioner.

[2] The title of the project as supplied by the applicant is 'Precision fermentation of alternative proteins'.

Risk assessment

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

Credible pathways to potential harm that were considered included exposure of people or other organisms to the GM yeast, and the potential for persistence or dispersal of the GM yeast. Potential harms associated with these pathways included allergenicity to people, and environmental harms due to the potential for the GM yeast to spread in the environment.

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

The principal reasons for the conclusion of negligible risks are that the GM yeast 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 GM yeast.

Risk management

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

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a limited and controlled release, the licence includes limits on the location and duration of the release. Controls are included to prohibit the use of the GM yeast in human food and animal feed, to minimise dispersal of the GM yeast from the production facility, to transport GM yeast in accordance with the Regulator's guidelines, and to destroy GM yeast at the end of the protein production process.

In addition, there are several general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements which include an obligation to report any unintended effects.